

ANNUAL REVIEW OF PHYSIOLOGY

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PREFACE

With this twenty-first volume the *Annual Review of Physiology* reaches its majority. Those of us in charge at present, pausing to note the attainment of this milestone, feel that we represent the generality of physiologists in greeting and thanking those to whom the *Annual Review* owes its origin: chiefly, Dr. J. Murray Luck, its first Editor, and the then Chairman of the Board of Publication Trustees of the American Physiological Society, Dr. Walter J. Meek. Born into a society about to be torn by war, it has matured in a period of almost explosive growth of the physiological sciences. The form and policies adopted at its inception, however, have remained essentially unaltered. Their effectiveness through the years is a testimony to the wisdom of the founders.

The delightful prefatory chapter of this notable year appropriately comes from Professor A. V. Hill. It is adapted from the first chapter of his "slowly growing" monograph on "The Heat Production of Muscle and Nerve". The frontispiece is from a portrait by H. Andrew Freeth, A.R.A., 1957, in the possession of King's College, Cambridge. Professor Hill comments:

The ancient umbrella discreetly figured may provoke inquiry. It was posted above my apparatus to protect it from the recurrent overflow of water from the laboratory above. Hitherto it has been effective, chiefly because of its educational value on my colleagues, who have thereby been reminded not to let their sinks overflow and their filter pumps burst.

A few notes about the other chapters in the volume are in order. Those of Drs. Eliassen and Pittendrigh in Comparative Physiology failed to become available. In the emergency Dr. A. C. Giese (incidentally one of our Associate Editors) provided an excellent chapter on reproductive cycles in invertebrates. The summary of Russian endocrinological and metabolic studies completes the series of such chapters which the *Annual Review* has been publishing with the support of the National Science Foundation. Since much of the Russian work is now readily available in translation, authors of the regular chapters will be encouraged and aided in reviewing Russian contributors in the same manner as those from the rest of the world.

Again we are pleased to thank our contributors for the time and effort they have so generously donated, our devoted and efficient editorial assistant, Mrs. Joann Huddleston, and our long-suffering printers, the George Banta Company, who have overcome manifold obstacles in the preparation of this volume.

J. M. B. F. A. F.
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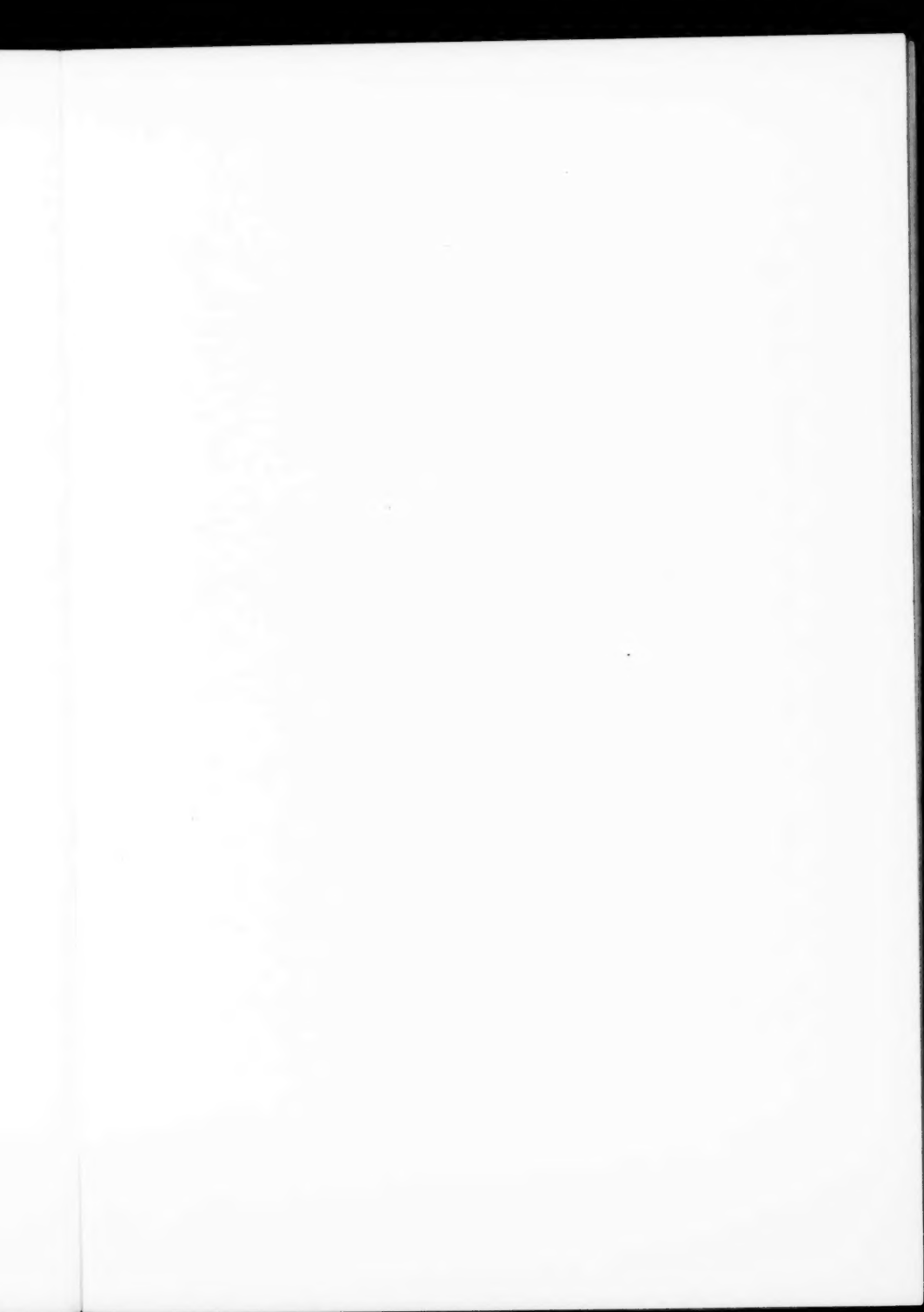
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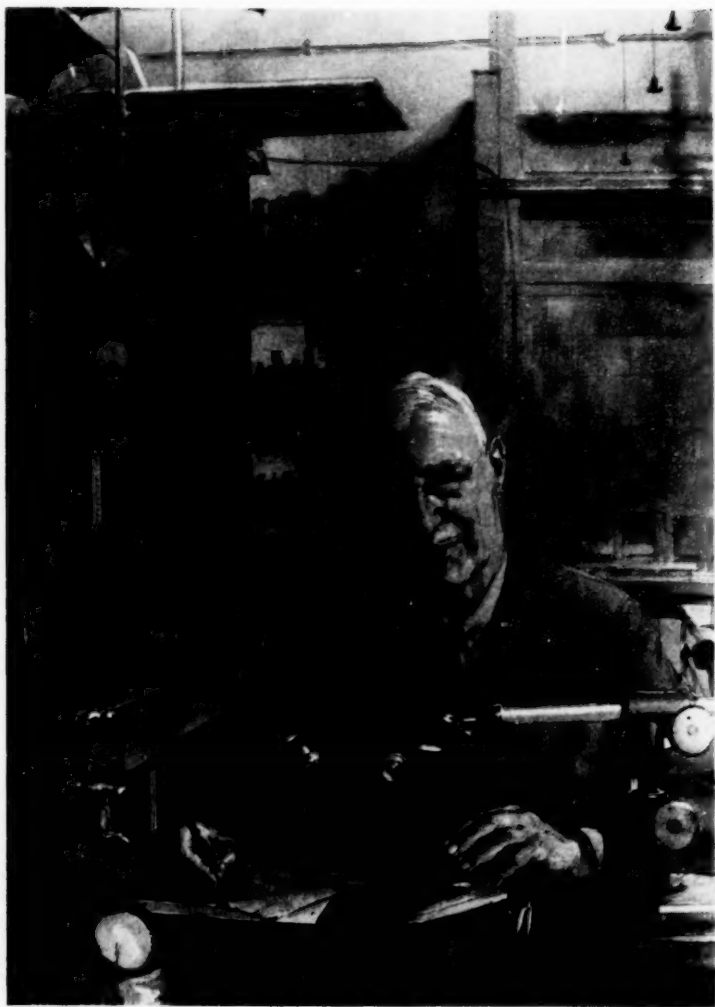
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A. V. HILL, from a portrait by H. Andrew Freeth, A.R.A., 1957,
in the possession of King's College, Cambridge.

THE HEAT PRODUCTION OF MUSCLE AND NERVE, 1848-1914¹

THE FIRST CHAPTER OF A FUTURE MONOGRAPH

By A. V. HILL

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In 1848 Helmholtz² published the first recorded experiments on the heat production of isolated muscle (30). Using three thermocouples in series with a galvanometer, he measured the rise of temperature in prolonged contractions of the muscles of a frog's leg. He was then 27 years old and still in the Prussian Army Medical Service, though he was allowed to retire later in the same year. In 1847 he had published his famous monograph *Ueber die Erhaltung der Kraft* (29), one of the epoch-making scientific contributions of the nineteenth century; though addressed to physicists, this had been prepared largely in consultation with physiologists, particularly his friend E. duBois-Reymond. The measurements of muscle heat had clearly been undertaken while his mind was revolving on the conservation of energy.

Helmholtz's paper in 1848 recorded also the first attempt to measure the heat production of stimulated nerve. Contrary to his expectation he could detect nothing; the sensitivity of his instruments was only about one thousandth, as we know now, of what was needed. He published no further experiments on either subject, though in 1850 he was the first to determine the velocity of conduction of a nerve impulse (31, 32). In 1851 he invented the ophthalmoscope, and, as professor of physiology successively at Königsberg, Bonn, and Heidelberg, he devoted himself mainly to vision and hearing. In 1871 he was called to the chair of physics in Berlin, and in 1888 was appointed also President of the newly formed Physikalisch-Technische Reichsanstalt at Charlottenburg. But by his early work on muscle heat production he had lighted a flame which, with a latent period of 15 years, burnt brightly in Germany till the end of the century.

Fifty years later, in June 1898, at about the time when this German work on heat production was coming to an end, a paper (17) of 90 pages (with 47 figures!) on *The Survival Respiration of Muscle* was published in the *Journal of Physiology*. Its author, Walter Morley Fletcher,³ nearly 25 years old, was then a medical student at St. Bartholomew's Hospital in London, where he had gone after election in 1897 to a fellowship at Trinity College, Cambridge. His research had started in 1895; he had chosen for himself to work on the

¹ The numbers in superior position in this chapter refer to the footnotes, which are mainly biographical.

² Helmholtz, Hermann (1821-1894). See Koenigsberger, L., *Hermann von Helmholtz* (Welby, F. A., Transl., Clarendon Press, Oxford, Engl., 1906); A.W.R. *Proc. Roy. Soc. (London)*, 59, xvii-xxx (1895-96).

³ Fletcher, Walter Morley (1873-1933). See Elliott, T. R., *Obituary Notices Roy. Soc.*, 1, 153-63 (1933); and Fletcher, M., *The Bright Countenance* (Hodder & Stoughton, London, Engl., 1957).

chemistry of muscle, which was outside the current interest of the Cambridge physiological laboratory of those days. Probably, however, Michael Foster⁴ had had something to do with it; certainly Foster's thoughts must have been on physiological chemistry, for in 1898 he invited F. G. Hopkins⁵ to Cambridge. Moreover, Fletcher told a story that, when he once inquired whether there was really anything more to be got out of chemistry for physiology, Foster rolled his great beard up with both hands over his mouth and emitted his characteristic chuckle.

Foster's answer may have helped to determine Fletcher's choice, and so to decide the sequence of research on the chemistry of muscle, at Cambridge and elsewhere. Doubtless Fletcher had other reasons. Perhaps a lively interest in athletics had biased him towards muscle; one reason may have been a leaning towards chemistry derived from his father; certainly, however, his direction was partly a result of the guidance of a botanist, F. F. Blackman,⁶ whose apparatus for measuring the respiration of plant leaves he borrowed and adapted for muscles. A photograph of Fletcher with that apparatus, in the physiological laboratory at Cambridge in 1897, is given in Maisie Fletcher's book, *The Bright Countenance*.³

As W. B. Hardy wrote in 1933, after Fletcher's death,

the subject was then stagnant, it was lost in all sorts of dead ends. . . . Fletcher gave it new life. Taking one thing with another we know more [today] of muscle and especially of the chemistry of movement than of any other form or activity of living matter. Origins are as difficult in science as in literature. Here, however, it is safe to credit Fletcher with that first step that counts.

Thus, in 1895 a young man of 22 took the first step that started off afresh an activity which is going on today wherever physiologists and biochemists are at work. Spallanzani (1800), Liebig (1850), Hermann (1870), Pflüger (1880) and many others had worked on the chemistry of muscle; but it had been served by inadequate methods and sterilized by fanciful theories embodied in words like "inogen" and "biogen"—hypothetical "giant molecules within which inscrutable chemical changes took place" (Dale⁶). In 1900 Fletcher got back to his muscles, having completed his medical examinations; in 1902 he published two papers (18, 19) on the influence of oxygen on their respiration, and in 1904 another (20) on their osmotic properties in fatigue and rigor.

Since 1898 Hopkins had been in Cambridge, and the community between them, evident in their close collaboration later in the work of the Medical Research Council, must have led Fletcher to discuss his problems with

⁴ Foster, Michael (1836–1907). Foster, a pupil of Sharpey, in 1870 had taken the gospel of physiology from University College, London, to Cambridge and was professor there from 1883 to 1903. See Langley, J. N., *J. Physiol. (London)*, **35**, 233–46 (1907); and Gaskell, W. H., *Proc. Roy. Soc. (London)*, **B80**, lxxi–lxxxi (1908). Gaskell's paper is a very striking tribute to Foster's influence.

⁵ Hopkins, Frederick Gowland (1861–1947). See Dale, H. H., *Obituary Notices Roy. Soc.*, **6**, 115–45 (1948).

⁶ Blackman, Frederick Frost (1866–1947). See Briggs, G. E., *Obituary Notices Roy. Soc.*, **5**, 651–58 (1948).

Hopkins. Indeed, those of us who were fortunate enough to know Hopkins always wanted to discuss our problems with him. About 1905 they began to work together on lactic acid in muscle, and in March, 1907, their famous paper (24) was published in the *Journal of Physiology*. Few papers in the history of physiology can have had so great an influence. Fletcher contributed his special knowledge of muscle, and his conviction that something rather momentous was waiting there to be found out; Hopkins provided the precise methods of chemical analysis; together they realized the importance, and the possibility, of arresting chemical changes in a living tissue before, and while, the destructive manipulations required for quantitative determinations were made. The work was done in Langley's laboratory (he had succeeded Foster in 1903), but he had no part in it except, as was his custom, to admire it from afar. Fletcher continued, amid many distractions, with the study of muscle, and three more papers (21, 22, 23) of his were published. However, in 1914 he became the secretary of the newly formed Medical Research Committee in London, and no more experimental work came from his own hands. Hopkins also never touched the subject again, but his experience with it must have helped to guide him in his later work on intermediary metabolism. In 1915, however, Fletcher & Hopkins jointly gave a Croonian Lecture (25) to the Royal Society on *The Respiratory Process in Muscle and the Nature of Muscular Motion*, an admirable summary, though impaired in its conclusions by their too generous acceptance of Parnas' results (see below). This, apart from the guidance and support they always gave to others, was their last direct contribution to the subject.

In November, 1909, Langley⁷ wrote me a letter proposing that I should "settle down to investigate the efficiency of cut-out frog's muscle as a thermodynamic machine." "There is," he said, "an especial problem suggested by Fletcher and Hopkins' work, as to the efficiency of the muscle

⁷ Langley, John Newport (1852-1925). See Fletcher, W. M., *J. Physiol. (London)*, **61**, 1-27 (1926); and Fletcher, W. M., *Proc. Roy. Soc. (London)*, **B101**, xxxiii-xli (1927).

From 1894 until his death in 1925, Langley owned and edited the *Journal of Physiology*. That he felt very keenly about the decencies of scientific publication is shown by the following remarks that exploded at the end of his presidential address in 1899 to the Physiology Section of the British Association. They are not less pertinent today, unless editors have abandoned hope.

"I am tempted, before ending, to make a slight digression. Those who have occasion to enter into the depths of what is oddly, if generously, called the literature of a scientific subject, alone know the difficulty of emerging with an unsoured disposition. The multitudinous facts presented by each corner of Nature form in large part the scientific man's burden today, and restrict him more and more, willy-nilly, to a narrower and narrower specialism. But that is not the whole of his burden. Much that he is forced to read consists of records of defective experiments, confused statement of results, wearisome description of detail, and unnecessarily protracted discussion of unnecessary hypotheses. The publication of such matter is a serious injury to the man of science; it absorbs the scanty funds of his libraries, and steals away his poor hours of leisure."

working with and without oxygen. . . . Once started there are plenty of further experiments to do and the question is a very important one for muscle physiology." "It would," he added, "be an advantage that Fletcher and Hopkins have done a good deal of work closely connected with this, so that you would have people interested in the subject to talk it over with."

I was then in the state of exaltation and relief that succeeded my last examination, Part II of the Tripos in Physiology, following Part I in Mathematics (the latter, in those days, with its Senior Wrangler and all, was rather like the Derby). After that nothing seemed too difficult, but fortunately there were plenty of people about to keep one on the rails. Indeed in the Cambridge Physiological Laboratory of that time, apart from one's contemporaries,⁸ there were more physiological giants to the square yard than in any other laboratory before or since, not only because there were very few square yards but also because there were so many giants.⁹ And within easy distance were plenty of other people available for advice and help.¹⁰ The environment is vividly described by Adrian in his contribution to Alys Keith-Lucas¹¹ book, and by Maisie Fletcher³ in hers.

Looking back on it now over half a century, it seems remarkable that Langley, who was very far from being a biophysicist or biochemist, should have proposed a subject of research so different from his own, yet so exactly fitted to the Cambridge atmosphere of that time and to my own inclinations: one moreover, which had been so fully exploited by the great German physiologists of the nineteenth century that nothing more might have seemed possible, when in fact the subject was ripe for development through the new ideas and methods then beginning to appear. And, no less remarkable, Langley produced, as though by magic, an apparatus (4) designed by Magnus Blix¹² of Lund, which he had acquired, goodness knows why, after some

⁸ Among others, V. H. Mottram, G. R. Mines, Geoffrey Evans, J. R. Marrack, H. Hartridge, George Winfield, E. D. Adrian, J. H. Burn, and R. A. Peters. These were not all there in 1909; several came later.

⁹ H. K. Anderson, J. Barcroft and his innumerable colleagues all shaking blood-gas apparatus; W. M. Fletcher, W. H. Gaskell, W. B. Hardy, F. G. Hopkins, J. N. Langley, and Keith Lucas.

¹⁰ For example, F. F. Blackman (botany), W. E. Dixon (pharmacology), H. O. Jones (chemistry), Bertram Hopkinson (engineering), C. G. Darwin (mathematics), T. B. Wood (agriculture), Horace Darwin and C. C. Mason (Cambridge Scientific Instrument Co.). The Cambridge Instrument Company, as it is now called, was started in the 1870's by A. G. Dew-Smith, a pupil and colleague of Michael Foster, largely for the purpose of making instruments for the physiological laboratory. Most of the earliest instruments had physiological and biological applications: this special interest was strongly maintained till his death ten years later, by the appointment, in 1906, of Keith Lucas as a director of the Company.

¹¹ Lucas, Keith (1879-1916). See Darwin, H., and Bayliss, W. M., *Proc. Roy. Soc. (London)*, B90, xxxi-xlii (1919); Langley, J. N., *J. Physiol. (London)*, 51, 35 (1917); and Lucas, Alys, *Keith Lucas* (Heffer & Sons, Cambridge, Engl., 1934).

¹² Blix, Magnus Gustaf (1849-1904). See Tigerstedt, R., *Skand. Arch. Physiol.*, 16, 334-47 (1904).

international physiological congress (Blix died in 1904). This beautiful little instrument, a thermocouple and galvanometer in one, was sensitive enough, so Blix had told him, to allow the heat produced in a single muscle twitch to be read (though it lacked its galvanometer magnets, and provided for a time rather a puzzle as to how it worked). After that, Langley took no further part in the research, except to advise me to apply to the Royal Society for a grant, to display occasionally a friendly interest in the results, and to rewrite, and make me rewrite and re-rewrite, the papers which I gave him at intervals for the Journal. Many of my younger colleagues since must have suffered from the editorial peculiarities I thus acquired, though I never took as much trouble for them in rewriting their papers as Langley⁷ did for most of us.

There were nearly five years before the First World War and innumerable opportunities of talking it over with Fletcher and with Hopkins. For Fletcher had been my tutor at Trinity (a Cambridge tutor is *in loco parentis* to his pupils), and he became, as it were, an elder brother, while Hopkins' rats (with which he was discovering vitamins) lived in cages around my instruments in a cellar. The cellar was odorous and overcrowded, but inspiring—inspiring not only for its frequent contacts with Hopkins, but because Keith Lucas¹¹ worked on the other side of a partition there, where he was later joined by Adrian and J. C. Bramwell. Their only entry was past the rats and instruments, which led to frequent and, to me, invaluable discussion. Not least among the benefits occurred when Lucas lent me the revolving contact-breaker which, apart from its idleness during two world wars and its daily activity for 14 years with Hartree, I have used ever since. Characteristic of Lucas' craftsmanship is the fact that, after nearly half a century and still on loan, this simple device works better for its purpose of controlling time intervals, from milliseconds to seconds, than any instrument I have known. Even electronic merchants have been known, reluctantly, to admire it.

Langley's intuition was right, and the use of the myothermic method led soon to critical experiments on the effect of oxygen. These were not usually thought out in advance, they were certainly not "planned" as doctrinaires profess to plan research, they arrived quietly by noticing odd things that turned up, by trying to understand them, and then seeing how they could be used. For example, the deflection of a galvanometer resulting from heat produced by stimulating a live muscle was observed to last considerably longer than that caused by the heat (mainly physical) liberated by an excessive direct stimulus. The latter, a casual observation, led to the use of the "heating control" and the method of electrical calibration; the former prompted the recognition of the "recovery heat" (37, 38, 41, 42), since the longer-lasting deflection could not be explained except by a slow, continuing heat production. This delayed heat, moreover, was found to occur only in the presence of oxygen, and the total amount of it could be measured; it was about equal to the "initial heat" which appeared impulsively during contraction. This led to the conclusion, which Fletcher & Hopkins (24) had been inclined to favour in 1907, that the lactic acid liberated during con-

traction is not oxidized but resynthesized, restored to its initial state. This conviction was strengthened later by Peters' (48) rather exact calorimetric measurements of the heat produced in muscle by prolonged stimulation, and a comparison with the heat of combustion of lactic acid.

It is now known that the conclusion is not really so simple as we thought then; and today one would probably say that in such short contractions as mine no lactic acid at all was liberated but that, as a net result, only creatine phosphate was split (and later resynthesized with the aid of energy supplied by oxidation). But the recovery heat in the presence of oxygen, under all sorts of conditions, is still about equal to the initial heat, and its implications after the war of 1914-18 led to a long and happy connexion with Otto Meyerhof.¹³

Another critical result with oxygen was obtained by Viktor Weizsäcker¹⁴ who worked at Cambridge in 1914. Weizsäcker, at Heidelberg, had been occupied (55) with a comparison of mechanical work and oxygen consumption in frogs' hearts and also with the inhibiting effects of cyanide. At Cambridge he made the fundamental observation (56, 57) that the "initial" heat is independent of the presence of oxygen and is unaltered by a heavy dose of cyanide. The chemical reactions, therefore, which liberate energy for the primary process of contraction are altogether nonoxidative in character. This result has been amply confirmed since; even the detailed time-course of the initial heat production, not merely its total amount, is independent of the availability of oxygen. As a result of the war, and later of his interest in clinical neurology (*die geistige Bedeutung der Krankheit*), Weizsäcker almost abandoned physiology from 1914 onwards. However, his final contribution to it (at the age of 28) was a fundamental one; its merit lay in asking (in Heidelberg) the right question and then going where the technique was available to answer it.

Since Fletcher and Hopkins' work, there had been much discussion of whether the removal of lactic acid from strongly fatigued muscle was caused by its oxidation within the muscle cells, or by its resynthesis into some "lactic acid precursor". They had stated the problem themselves and given their tentative answer. J. K. Parnas had come to Cambridge in 1914, shortly before the war, in order, as he hoped, to decide the question by thermal measurements. He had recently shown at Strassburg [Parnas & Wagner (47)] that, under most conditions of activity and breakdown, frogs' muscles

¹³ Meyerhof, Otto (1884-1951). See Peters, R. A., *Obituary Notices Roy. Soc.*, 9, 175-200 (1954).

¹⁴ Weizsäcker, Viktor (1886-1957). He studied physiology with J. von Kries, medicine with L. von Krehl; and did early research on mechanical work and gas exchange in the frog's heart. In 1920 he went over to neurology in the Heidelberg clinic. He was the grandson and great-grandson of Schwabian theologians and this was reflected in his philosophical and moral approach to illness. In 1941 he was appointed to the Chair of Neurology at Breslau; when Breslau was lost to Germany in 1945 he was given a special chair in general clinical medicine at Heidelberg. In both wars he was a prisoner of the United States Armies.

exhibit parallel changes of lactic acid formation and carbohydrate loss. Under some conditions, however, the correspondence had failed, and Parnas concluded that the precursor of lactic acid was not carbohydrate but something compounded of it. At Cambridge he made parallel determinations, on "completely fatigued" frogs' gastrocnemii recovering in oxygen, (a) of the extra oxygen used in 20 hours recovery, and (b) in a calorimeter, of the extra heat liberated. He found (a) that the extra oxygen was sufficient to burn the lactic acid that disappeared and (b) that the extra heat was about half the heat of combustion. He concluded, first, that the lactic acid of fatigue is completely burnt and not rebuilt, and, second, that about one-half of the energy so liberated is stored as potential energy in the muscle.

Parnas' experiments, for so difficult an investigation, were made in a very short time and were ended by the outbreak of war, so that I had no opportunity of discussing them with him. He, as a German citizen, was interned (and later repatriated) while I was in the Army. The state of his "completely fatigued" muscles may have been abnormal, as compared with that of the very moderately stimulated muscles used in my experiments in 1912 and after, and their recovery took 20 hours as compared with the few minutes of mine. His extra oxygen and extra heat may have been attributable to processes other than the normal removal of lactic acid and so have been too large. At any rate, his conclusions were wrong. However, they were communicated to the Physiological Society (45) and published in greater detail later (46) in Germany. They were accepted by Fletcher & Hopkins (25) in their Croonian Lecture (and seriously affected their deductions), and, at first, by Meyerhof; but in 1920, on closer examination, Meyerhof (44) showed beyond doubt that in the recovery of muscles from fatigue the oxygen used, the heat produced, the lactic acid removed, and the carbohydrate reformed all fitted into a consistent scheme. When lactic acid disappeared under the influence of oxygen, only a third or a quarter of it was burnt (or a corresponding amount of carbohydrate); the rest reappeared in carbohydrate form.¹⁵

So the circle was completed, back to Fletcher and Hopkins' original

¹⁵ An entertaining personal story may be added of the conflict of conclusions between Parnas and Meyerhof. In July 1920 an "International" Congress of Physiologists was to be held in Paris, from which "enemy" scientists were to be excluded. In March 1920 Meyerhof had sent his results for publication to *Pflügers Archiv*, and he complained to me bitterly in a letter that he was not to be allowed to attend the Congress and report them, whereas Parnas, no longer a German "enemy" but now a Polish "ally", was intending to read a paper on his contrary findings of 1914, based on much less critical evidence than Meyerhof had obtained. Yet Parnas, in Cambridge from Strassburg in 1914, had been an open and vigorous supporter of German militarism, which Meyerhof had always deplored. In the event, however, neither of them went to the Congress, for Parnas was cut off in Warsaw by the Russian armies which had invaded Poland in July, and it was left to the Congress at Edinburgh of 1923, under the presidency of Sharpey-Schafer (who himself had lost two sons in the war) to become properly international again.

tentative explanation of their results, that lactic acid is not burnt during recovery but rebuilt. It had been difficult not to accept that view, anyhow, once the heat of formation of lactic acid in muscle was known and compared with its heat of combustion. Teleological arguments are notoriously dangerous, but it was difficult to believe that an important constituent of the muscle mechanism, containing a large amount of energy, had to be burnt every time it was produced, in order merely to get rid of it, thereby placing a heavy extra load on the respiratory and circulatory system. From the engineering standpoint, the design of muscles is not so stupid as that [Hill (43)].

The experiments discussed so far were made on isolated muscle; but, just when it was realized that in such muscle a considerable amount of heat is liberated in recovery after contraction, two papers appeared, by Barcroft¹⁶ and his colleagues, describing analogous results on whole, but anaesthetized, animals. In the first, Verzàr (54) showed that, after a rather long tetanus of a cat's muscle, excess oxygen continued to be used for several minutes; in the second, Barcroft & Piper (1), studying the extra oxygen consumption resulting from stimulation of the submaxillary gland of the cat, found that oxygen was used for some time after saliva had ceased to flow. They concluded

the oxygen appears to be used not in directly providing the energy necessary for the secretion of saliva, but rather for re-establishing the potential energy of the physical or chemical system which performs the complex function of secretion.

The phenomenon, in fact, is rather a general one: it is very evident after severe muscular exercise in man (where it led later to the concept of "oxygen debt"); it occurs in nerve after stimulation. It would be interesting to know whether there are exceptions to the rule that when physiological activity is provoked suddenly through a nerve, it is followed by a slower chemical process of recovery.

Let us return now to the earlier part of this story. After Helmholtz's original publication in 1848, there was an interval of 15 years during which nothing important appeared, then in the 1860's a long series of papers began. The greatest contributor was Adolf Fick,¹⁷ whose name, curiously enough, is perpetuated not in physiology but in physics, in Fick's Law of Diffusion which he announced in 1855 when he was 26 (11, 12). The German physiologists of that era were certainly accomplished physicists. But physiologists also should remember him, for he was the originator of terms they use every day, "isometric" and "isotonic" applied to muscular contraction (13, pp. 112, 131).

Fick was preceded by a few years in publication on muscle heat by Rudolf Heidenhain¹⁸ whose remarkable monograph (27) in 1864, when he

¹⁶ Barcroft, Joseph (1872-1947). He was Langley's successor, 1926 to 1937. See Roughton, F. J. W., *Obituary Notices Roy. Soc.*, **6**, 317-45 (1949).

¹⁷ Fick, Adolf (1829-1901). See Schenk, F., *Arch. ges. Physiol.*, **90**, 313-61 (1902); this article is reprinted in Fick, A., *Gesammelte Schriften*, **1** (Stahel, Würzburg, Germany, 1903).

was 30 years old, contained practically his first and last words, apart from controversy, on the subject. Heidenhain had originally expected that with a constant stimulus the total energy in a contraction, i.e., the sum of work and heat, would be constant too. He was astonished to find that when the initial tension was altered in an isometric contraction, or the work was altered by changing the load, the total energy changed too; that the muscle contained a "governor" by which the energy used was largely determined by length and load. With a technique which today would be regarded as primitive, with a sensitivity which gave only about seven scale-divisions for a muscle twitch (subject also to serious thermal disturbances, described as "negative Wärmeschwankungen"), he nevertheless arrived at a result which Fick (13, p. 179) described as "*eine der bedeutsamsten physiologischen Entdeckungen der Neuzeit*." Heidenhain even went so far as to test his conclusions chemically. If two similar gastrocnemii were similarly stimulated to 100 or 120 twitches under two different loads, great and small, so that one of them did much more work than its companion, the one doing the greater work gave a greater colour change to litmus. This might be a good class-demonstration today! In a final chapter on the theory of muscle force, he concluded that the processes that occur when a muscle is stimulated have a different nature and origin from those that cause a stretched rubber band to shorten. He was completely right, though the matter has often been debated since. Heidenhain's conclusions, and similar results obtained by Fick, were discussed by W. O. Fenn (10) in 1923 in an introduction to his own important work on the subject.

Unlike Heidenhain's, Fick's contributions to muscle heat, with those of his pupils, extended over many years. The results are described in his monograph (13) in 1882, in his *Myothermische Untersuchungen* (15) in 1889, and in his *Gesammelte Schriften* (16, Vol. 2). His best known pupil was Magnus Blix,¹² a Swede, who worked with him in 1880-81, and later devised the apparatus referred to on p. 4 above. Curiously enough, Blix published only two important papers (3, 4) on muscle heat (17 years apart, in 1885 and 1902), though there were several others on muscle elasticity. His paper in 1885 discussed the question whether, in muscular contraction, heat is transformed into work. It is strange that this question should ever have been debated seriously, though it was, again and again; for, if muscle were a heat engine, differences of temperature of at least 100°C. would have to exist within a muscle fibre, to explain an observed "thermal efficiency" of 25 per cent. His paper of 1902 contained a detailed account of technique and a long discussion of previous results. His epigrammatic conclusion that "*Länge macht Wärme*", which meant that the amount of energy liberated is determined by the "chemically active surface" during contraction, is far too simple a description of the true facts.

Apart from the work of Heidenhain, the chief conclusions (so far as they

¹² Heidenhain, Rudolf (1834-1897). He studied in Königsberg and Halle/Saale; was Assistant (1854-56) to E. du Bois-Reymond; Professor at Breslau (1859-97); chiefly known for his work (1867-97) on gland secretion. See Grutznher, P., *Arch. ges. Physiol.*, **72**, 221-65 (1898).

were correct) can be summarized as follows. (a) In a maximal twitch, the heat produced is about three millicalories per gram of muscle. (b) Direct stimulation and stimulation through the nerve give the same heat, provided that the mechanical response is the same. (c) In a tetanus, the longer a muscle is stimulated the greater is the total heat produced, but the rate of heat production decreases continually as stimulation continues. (d) In a tetanus, the heat production is independent of the frequency of stimulation, so long as the mechanical response is the same. (e) The work done in contraction is not derived from heat but directly from chemical reactions. (f) The ratio of work to total energy in contraction depends on the load and has a maximum value of about 0.3. (g) When a muscle is stimulated, the chemical processes providing mechanical work occur during the actual performance of that work. They do not create a store of potential (e.g., elastic) energy which can be used for doing work later on. (h) A muscle poisoned with veratrin, giving a prolonged contraction in response to a single shock, produces much more heat than a normal muscle. (i) A frog's muscle at rest shortens on warming, lengthens on cooling. (j) Conversely, when a muscle at rest is extended its temperature rises, when released its temperature falls. This seems a meagre harvest from the expenditure of so much effort, ingenuity and learning over so long; but a great deal has been omitted from the list which is now known to be wrong, or to have no special significance today. In fact, of the material included some was not very firmly established. A brief summary by Sanderson (50) appeared in *Schäfers Text Book of Physiology* in 1900, and a rather detailed discussion and criticism of Fick's experiments and arguments on the mechanical efficiency of muscle were given in my paper (39) in 1913 under that title. My own experiments and conclusions of that time are now of little value, because of technical errors which were overcome only many years later, because their discussion was obsessed by the false idea that during activity a muscle produces elastic potential energy which can be turned into work if the mechanical conditions allow, and because of failure to realize that an ordinary "isometric" contraction is not nearly isometric so far as the muscle fibres are concerned. But my criticism was mostly valid.

The most substantial account (26) of the whole of the earlier work is that published by Otto Frank¹⁹ in 1904. Frank himself had never worked on the subject; but the fact that he came to it without personal bias, together with his critical integrity and unrelenting reliance on precision in experiment and argument, may have helped him to sort out the pertinent results and fruitful ideas from an alarming mass of conflicting evidence and doubtful conclusions. The same obstinate integrity led him 30 years later into conflict with the Nazis. That he was not unsympathetic to those whom he regarded as the chief contributors to the subject is shown by some concluding remarks:

¹⁹ Frank, Otto (1865-1944). See Wezler, K., *Z. Biol.*, **103**, 91-122 (1950). This is a notable biography.

It has been my essential purpose to ensure that the fundamental ideas and the exact methods of these authors should not be destined to oblivion. In that sense these pages are dedicated to their memory.

Yet he had written in his introduction:

When I undertook this task it did not seem so difficult as I have since realized it to be. One became frankly dismayed at the appalling uncertainty which still affects all aspects of the subject. . . . It was essential not to restrain one's criticism. . . .

Many of the questions which had been asked could not be answered by methods available then or for many years after, some needed more precise formulation in chemical or physicochemical terms, others had to wait for better knowledge of the plain mechanics of contraction. Fick himself had confessed in 1884, in the introduction to his paper (14) *Myothermische Fragen und Versuche*,

as the title shows I lay at least as much weight on asking the questions and discussing them as on the actual results of the experiments described.

In his final chapter Frank discussed one of Fick's questions,

Do the thermal phenomena in active muscle give an answer to the problem of whether two separate chemical processes are involved, one in contraction, the other in relaxation?

The question was well put, and has a very modern sound; but with the evidence at hand, and with the methods available, no answer could be given till many years had passed. Frank's final sentence, in this supremely good survey, was:

Perhaps—and that is the highest aim of my efforts—future investigation can derive a stimulus from this review.

I discovered Frank's paper in 1911, 167 pages of long and elegant German sentences, at a time when my knowledge of German was very meagre: and in tribute to his memory I can claim emphatically that it did.

In 1908 Karl Bürker²⁰ published in *Tigerstedt's Handbuch* a very full account (5) of *Methoden zur Thermodynamik des Muskels*, describing the methods used by all previous investigators. This makes it unnecessary to refer here to the methods used before 1908, and they have in fact been altogether superseded by others developed since. Muscle heat is a subject in which the closest attention to experimental technique, and a real understanding of it, are necessary. It is dangerously easy to get beautiful "thermomyograms"; the problem is to know what, if anything, they really mean, to transform them into absolute units of heat and time, and to be sure what errors affect them. The chief weaknesses of the older methods were the following. (a) They were extremely slow, so that it was scarcely possible, and

²⁰ Bürker, Karl (1872–1957). He studied in Tübingen, became (1904) a.o. Professor there, and then in 1917 was called to the chair of physiology in Giessen; in 1945 he retired to Tübingen. His best known work was on the heat production of muscle and on the physiology of blood.

in fact no attempt was made, to determine the time-course of the heat production during and after a contraction. Investigators were content with a single maximum deflection, representing more or less nearly the total heat produced up to the time when the deflection was read. The essential contribution in more recent times has been to provide a picture of the whole time-course of the heat, in relation to stimulus and mechanical response; and most of our knowledge today results from bringing in that other dimension of time. (b) No accuracy was possible in determining either the rate of heat production at rest, or the heat produced over a long interval, e.g., in recovery. These calculations required a degree of thermal stability which could not be attained without a far-reaching redesign of the instruments. (c) Calibration in absolute units of heat was inadequate; no account was taken either of the heat capacity of the instruments, or of heat loss up to the moment when a deflection was read. Accurate calibration is essential in comparing thermal and mechanical effects. (d) Close and consistent contact of muscle with thermopile is necessary if true records are to be obtained, and this contact must be made over an area large enough to give a reasonable average of the change of temperature. Many of the muscles used, and the thermopiles, did not meet this requirement. (e) When muscles are allowed to move, as in doing mechanical work, pre-existing differences of temperature along their length may cause grave errors impossible to allow for or sometimes even to detect. Such errors are avoided today by a special provision in the instruments, also by placing the thermopile and muscle in a container at constant temperature. (f) The electrical insulator shielding the thermopile from direct contact with a wet muscle either had to be thick, thus introducing delay and unnecessary heat capacity, as if it was thin, bad electrical leaks might occur. With modern insulating materials the situation is altogether easier.

Moreover subsidiary methods, e.g., of stimulating, mechanical recording, and timing, have added a new order of facility to the complex business of combined mechanical and thermal observation, while a much better knowledge of the physiology of muscle not only can guide one to asking the right questions, but can provide a consistency and reliability comparable with that of more ordinary physical measurements.

Returning now to Bürker, his 1908 paper led me in 1911 to spend two months with him in the pleasant little university town of Tübingen. There I learnt about his methods of constructing thermopiles and had the great good fortune to meet Friedrich Paschen,²¹ the professor of physics and a famous contributor to infrared spectroscopy. Later, from 1924 on, Paschen was Director of the Physikalisch-Technische Reichsanstalt in Berlin until he was dismissed by the Nazis in 1933 and replaced by the notorious Johan-

²¹Paschen, Friedrich (1865-1947). In 1888 he received his doctorate in Strassburg with Kundt and Kohlrausch; in 1900, he became Professor of Physics at Tübingen; in 1924, director of the Physikalisch-Technische Reichsanstalt, Charlottenberg; and in 1933 he was dismissed by the Nazis. "A giant among spectroscopists". See the charming biography by Tolansky, S., *Nature*, **159**, 529-30 (1947).

nes Stark; in 1928 he was Rumford medallist of the Royal Society. With characteristic good nature, and often with loud shouts of laughter, Paschen taught me about his fine moving magnet galvanometers, and he allowed me to carry away in my bag (for 80 marks) a beautiful iron-shielded instrument made in his laboratory. This galvanometer lived three and one-half years in my cellar, among the cages of Hopkins' rats, and in 1914 was used by Weizsäcker and Parnas to make their experiments too. Paschen's generous advice was available again, from 1920, when we started constructing galvanometers like his in England. Bürker later published another paper (6) on methods, but it contained little new. The kindly help of Bürker & Paschen in 1911 gave a start (35, 38) to the improvements of technique which have continued since for many years, largely at other people's hands: Weizsäcker's in 1914, Fenn's in 1922, Hartree's from 1919 to 1933, and Downing's from 1920 to the present day. But that is another story which will be told later.

Rutherford once remarked to me, apropos of nothing in particular, "I've just been reading some of my early papers; and when I'd read them for a bit I said to myself 'Ernest my boy, you used to be a damned clever fellow.' " I could not imitate Rutherford's charming egotism; but in rereading for the present purpose those of my own papers which come into the period of this Chapter, written between the ages of 24 and 27, I have been interested to find how much in them, of ideas and method, has proved to be the basis of what has been done since. That, perhaps, is not an unusual experience. Of course it is quite untrue that most of the best original research is done by quite young people. I should myself have been a very unhappy man had I been unable to return at 59 to the work which was suddenly cut short by the Second World War at 53. But it is true that the lines of one's original thinking are generally laid down when one is quite young. However, if one is lucky with the subject and one's colleagues, and with the manner in which an improving technique continues to open up new ways of testing ideas, then one's actual published work before the age of 30 may look naive and unfinished in comparison with that of later years. That at least is my experience, but I have been very lucky.

Another thought that often comes to one's mind as one reads one's own, or anyone else's, earlier papers is the question, why on earth didn't we recognize then what is perfectly apparent now? Why did it take me, for example, 25 years to discover the simple device (a "protecting" region) by which the serious technical error caused by movement of a muscle over a thermopile can be avoided? Much effort and printer's ink would have been saved had this been introduced in 1912 instead of 1937, and the true relations between heat, work, load, and shortening could have been settled many years earlier. Or again, why did it take so long to realize that the series elastic component of muscle (or its recording devices!) exerts a dominating influence on the observed form of a contraction; that an "isometric" contraction is not isometric at all so far as the fibres are concerned; that the mechanical work performed in it is not zero but may be not far short of a maximum; that the emergence of the "active state" after a stimulus is very rapid, and does not

follow at all the ordinary form of a contraction? Why do ideas, later seen to be obvious, often come so slowly, to young and old alike, to one's colleagues as well as oneself? I have no answer to suggest, but perhaps by keeping the question continually in mind one may help to obviate the need of one.

In my papers of 1910-1914, there is much that is now known to be wrong, some of the "facts" and several of the assumptions and conclusions. But there are some that are right: apart from those already mentioned, the general idea that a slower twitch is associated with the more economical maintenance of a tetanus; the laying of the ghost of intramolecular oxygen; the emphasis on physical chemistry and thermodynamics. The things that are right—and pertinent—have since been confirmed and extended by better methods; they will be discussed later in my monograph and there is no need to refer to them further here. But some people may still wish to see the original papers, and for that purpose Table I may be useful. They may find occasional flashes of amusement, e.g., in the provocative claim (34, p. 43) by a

TABLE I
INDEX TO TOPICS IN PAPERS OF A. V. HILL ON HEAT
PRODUCTION OF MUSCLE
(Papers are those of A. V. Hill except where otherwise noted.)

Topic	Reference numbers
Absolute values of the heat, for comparison with work and tension	(39, 40)
Heat in prolonged contractions: influence of duration, frequency of stimulation, temperature, previous activity	(34, 40)
Effect of length and load	(34, 40) and Evans & Hill (9)
Time at which heat is produced	(33, 34, 38)
Recovery heat	(37, 38, 41, 42)
Initial process nonoxidative	(37) and Weizsäcker (56, 57)
Intramolecular oxygen	(38)
Heat production of anaerobic survival, fatigue and rigor, contrary to Dybowski & Fick (8)	36) and Peters (48)
Physical chemistry and thermodynamics	Appendix (36, 39)

youngster of 24: "a complete investigation of these facts will give us more real insight into the nature of the muscular machine . . . than any theories of contraction ever founded by ingenious minds upon insufficient knowledge." Langley must have liked that sentence, or he would never have allowed me to finish my paper with it; but it nearly led to blows, at a meeting of the Physiological Society, with the author of one such theory. In fact I have always been ready to defend the proposition that all theories of contraction are wrong—including any of my own. It seems to be a general characteristic of the family, but perhaps some day a viable hybrid will be produced.

Langley had told me in 1909, "there are plenty of further experiments to

do." It seems strange, looking back, that so many of them could have been made in less than five years; but they are not finished yet, and it may be worth while, before ending this Chapter, to include a few general reflections on the "philosophy" of them that emerged, chiefly later on, as knowledge and experience grew, as technical developments occurred; and as other lines of progress were disentangled. Let us assume that the primary purpose of biophysics and biochemistry is to relate observed physiological phenomena to physical and chemical events and causes at a molecular level. Muscular contraction is among the most evident of such phenomena, and for exact experimentation it has two special advantages: (a) that its end results, force, movement and work, can be accurately and rapidly measured in absolute terms, and (b) that they can be elicited at will by an electric stimulus. An obvious and immediate interest is to relate the mechanical occurrences, particularly the work, to the exchanges of total energy involved. That energy, however, is derived from chemical change; and a problem of equal importance, but much more troublesome, is to connect the mechanical events with the chemical ones.

The difficulty is that chemical methods are usually extremely slow and insensitive compared with those of recording mechanical events: e.g., work measured in tens or hundreds of ergs is easily recorded without significant delay, but the chemical reaction which produced it might involve only thousandths of a microgram of substance. Even if chemical changes of such magnitude could be measured, it is scarcely conceivable that this could be done in the small fraction of a second occupied by the mechanical event, particularly since most chemical estimations in living cells involve the destruction of the material itself. The only method usually available is to measure the chemical changes that accrue during a series of contractions; and this involves the danger, almost the certainty, that the finer and more fundamental details will be obscured or distorted by examining only the end products, not the primary processes, of the reaction. The astonishing thing, indeed, is that biochemistry has gone as far as it has, in its analysis of intermediate events.

For measuring heat, however, the methods available are very sensitive, and can be made very rapid—not indeed as sensitive and rapid as those for recording mechanical changes, but incomparably better in both respects than usual chemical methods. The heat is related to the chemical exchanges, not always indeed in a specific way, but at least in a manner which provides a firm outline that must not be overstepped and can be filled in as knowledge accrues. In nerve, where no aid is derived from mechanical manifestations of activity, and the electric change is the only immediate sign, the heat provides a valuable further object of study, for comparison with ionic exchanges, and eventually, when they are successfully measured, with chemical exchanges too.

To summarize, therefore, the special value of the heat as an object of research and an index of activity in muscle and nerve is its intimate relation to the mechanical and chemical changes involved, and the sensitivity and speed of the methods available. Measurement of the heat admittedly does not point unequivocally to the actual chemical processes that occur,

but it does provide a framework into which they must be fitted.

For many years attempts were made to measure the heat production of stimulated nerve. Helmholtz (30) in 1848 had failed to find any in frogs' nerves, but he could read only to $0.002^{\circ}\text{C}.$; about 1000 times this sensitivity was needed. Valentin (53) in 1863 and Schiff (51) in 1869 claimed to have obtained positive results. However, Valentin's results must certainly have been caused by the Joule's heat of the stimulating current (which is not easy to avoid), while Schiff's were not quantitative, and no precautions against leak of heat from the stimulus were described. Heidenhain (28) in 1868, with about ten times Helmholtz's sensitivity, again obtained a negative result. In 1890 Humphrey Rolleston²² in Cambridge turned to the subject (49); it is an intriguing question what led him to it, in view of his later clinical interests. He used a platinum resistance thermometer, with which he could read to $0.0002^{\circ}\text{C}.$, about the same as with Heidenhain's thermopile. Taking care to avoid the Joule's heat of the stimulating current, he found no measurable heat produced by the nerves; he needed 100 times the sensitivity, which no resistance thermometer could possibly have provided. In the following year, also at Cambridge, G. N. Stewart (later Professor of Physiology at Western Reserve University) tried again (52), with similar instruments, on mammalian nerve; he also was unsuccessful. Finally in 1897 Max Cremer (7), thinking that nonmedullated nerves might give more heat than medullated ones (which later events confirmed), tried the olfactory nerves of pike, carp, and barbel. Reading to $0.0001^{\circ}\text{C}.$, he concluded that the heat, if any, was certainly less than the disturbance caused by the Joule's heat of the stimulating current.

Why did people go on trying to measure the heat production of nerve, in spite of repeated failure? Chiefly, I suppose, in order to settle the question of whether the nerve impulse is the sort of physical wave in which the whole of the energy for transmission is impressed on the system at the start. Various properties of nerve, superficially at least, favoured this view, particularly the classical demonstration of its "infatigability". Against it was the existence of an absolute refractory period, during which, after the passage of one impulse, a second one cannot be carried; in this property the nerve impulse is unlike any physical wave in which the energy is supplied at the start. If it could be shown that heat really was produced all along a nerve during transmission, then the purely physical theory of conduction would be untenable. A distributed relay system would be required, with energy derived presumably from chemical change.

With such ideas in mind, though not so clearly as today, in 1912 I made another unsuccessful attack (35) on the problem, using the improved thermoelectric instruments constructed after my visit to Bürker and Paschen in 1911. Once more, nothing was found, though success was really rather near. The thermal stability was poor, and the real sensitivity must have been considerably less than I estimated, because of factors not properly realized

²² Rolleston, Humphrey Davy (1862-1944). He was a physician; Fellow of St. John's College, Cambridge; President, Royal College of Physicians, 1922-26; Regius Professor of Physic, Cambridge University, 1925-32.

at that time. Making a rough allowance now in retrospect, what was really shown was that 600 impulses did not cause a heat production of more than about 3×10^{-8} cal. per gm. or for one impulse 5×10^{-8} cal. per gm. As a matter of fact heat is produced, and I had very nearly measured it; for 20 years later the heat per impulse at the same frequency was found to be about 6×10^{-8} cal. per gm. Had the limb nerves of spider crabs been used instead of the sciatic nerves of frogs, the heat would have been large and obvious.

Bayliss was quick to see the implications of this new failure, at a much higher sensitivity, to detect any heat and in 1915 in his *Principles of General Physiology* (2, p. 378) he wrote:

The result makes it impossible to suppose that any chemical process resulting in an irreversible loss of energy can be involved [in the transmission of a nerve impulse], and indicates that a reversible physicochemical one of some kind is to be looked for.

I had made originally a similar claim myself, but when the excitement died down I could not really believe it, particularly in face of the absolute refractory period and its consequences. Many things occur in nerve which are quite unlike what happens in the transmission of an ordinary physical wave, while the supposed physicochemical changes were so rapid that they were rather unlikely to go on "reversibly" in such a medium as nerve. Anyway at intervals, after the war of 1914-1918, I went on trying, now with Downing's help; and in 1926 at last positive results were obtained.

LITERATURE CITED

1. Barcroft, J. and Piper, H., *J. Physiol. (London)*, **44**, 359-73 (1912)
2. Bayliss, W. M., *Principles of General Physiology* (Longmans, Green & Co., London, Engl., 1915)
3. Blix, M., *Z. Biol.*, **21**, 190-249 (1885); in Fick, A., *Myothermische Untersuchungen*, 195-248 (Bergmann, Wiesbaden, Germany, 1889)
4. Blix, M., *Skand. Arch. Physiol.*, **12**, 52-128 (1902)
5. Bürker, K., *Handb. (Tigerstedt) physiol. Meth.*, **2**(3), 1-86 (1908)
6. Bürker, K., *Arch. ges. Physiol.*, **174**, 282-323 (1919)
7. Cremer, M., *Münch. med. Wochschr.*, **44**, 280-81 (1897)
8. Dybowski, W., and Fick, A., *Vierteljahresschrift der Naturforsch. ges. in Zurich, Jahrgang 1867* (Braumüller, Wien, Austria, 1869); cited by Fick, A., *Myothermische Untersuchungen*, 53-57 (Bergmann, Wiesbaden, Germany, 1889); also Fick, *Gesammelte Schriften*, **2**, 177-97 (Stahel, Würzburg, Germany, 1903)
9. Evans, C. L., and Hill, A. C., *J. Physiol. (London)*, **49**, 10-16 (1914)
10. Fenn, W. O., *J. Physiol. (London)*, **58**, 175-203 (1923)
11. Fick, A., *Poggendorfs Ann.*, **94**, 59-86 (1855); also *Gesammelte Schriften*, **1**, 208-30 (Stahel, Würzburg, Germany, 1903)
12. Fick, A., *Z. rat. Med.*, **6**, 288-301 (1855); *Gesammelte Schriften*, **1**, 231-43 (Stahel, Würzburg, Germany, 1903)
13. Fick, A., *Mechanische Arbeit u. Wärmeentwicklung b.d. Muskelthätigkeit* (Internationale wissenschaftliche Bibliothek, 51, Brockhaus, Leipzig, Germany, 1882)
14. Fick, A., *Myothermische Untersuchungen*, 249-70 (Bergmann, Wiesbaden, Germany, 1884); also *Gesammelte Schriften*, **2**, 295-316 (Stahel, Würzburg, Germany, 1903)

15. Fick, A., *Myothermische Untersuchungen* (Bergmann, Wiesbaden, Germany, 1889)
16. Fick, A., *Gesammelte Schriften*, **1**, **2**, **3**, **4** (Stahel, Würzburg, Germany, 1903)
17. Fletcher, W. M., *J. Physiol. (London)*, **23**, 10-99 (1898)
18. Fletcher, W. M., *J. Physiol. (London)*, **28**, 354-59 (1902)
19. Fletcher, W. M., *J. Physiol. (London)*, **28**, 474-98 (1902)
20. Fletcher, W. M., *J. Physiol. (London)*, **30**, 414-38 (1904)
21. Fletcher, W. M., *J. Physiol. (London)*, **43**, 286-312 (1911)
22. Fletcher, W. M., *J. Physiol. (London)*, **47**, 361-80 (1913)
23. Fletcher, W. M., and Brown, G. M., *J. Physiol. (London)*, **48**, 177-204 (1914)
24. Fletcher, W. M., and Hopkins, F. G., *J. Physiol. (London)*, **35**, 247-309 (1907)
25. Fletcher, W. M., and Hopkins, F. G., *Proc. Roy. Soc. (London)*, **B89**, 444-67 (1917)
26. Frank, O., *Ergeb. Physiol.* **3**(2), 348-514 (1904)
27. Heidenhain, R., *Mechanische Leistung, Wärmeentwicklung und Stoffumsatz bei der Muskeltätigkeit* (Breitkopf u. Härtel, Leipzig, Germany, 1864). This book is rarely accessible, but reference to it occurs in Meissner, G., in *Ber. ü.d. Fortschritte Anat. u. Physiol. Abstr.*, 427-33 (1864) and Grützner, P., *Arch. ges. Physiol.*, **72**, 47 (1898) (biographical notice of Heidenhain)
28. Heidenhain, R., *Stud. physiol. Instit. Breslau*, **4**, 248-50 (1868)
29. Helmholtz, H., *Ueber die Erhaltung der Kraft* (Reimer, Berlin, Germany, 1847); reprinted in *Ostwald's Klassiker* (Engelmann, Leipzig, Germany, 1907)
30. Helmholtz, H., *Arch. Anat. u. Physiol.*, 144-64 (1848)
31. Helmholtz, H., *Arch. Anat. u. Physiol.*, 71-73 (1850)
32. Helmholtz, H., *Arch. Anat. u. Physiol.*, 199-216 (1852)
33. Hill, A. V., *J. Physiol. (London)*, **40**, 389-403 (1910)
34. Hill, A. V., *J. Physiol. (London)*, **42**, 1-43 (1911)
35. Hill, A. V., *J. Physiol. (London)*, **43**, 433-40 (1912)
36. Hill, A. V., *J. Physiol. (London)*, **44**, 466-513 (1912)
37. Hill, A. V., *J. Physiol. (London)*, **45**, xxxv-xxxvii (1912)
38. Hill, A. V., *J. Physiol. (London)*, **46**, 28-80 (1913)
39. Hill, A. V., *J. Physiol. (London)*, **46**, 435-69 (1913)
40. Hill, A. V., *J. Physiol. (London)*, **47**, 305-24 (1913)
41. Hill, A. V., *J. Physiol. (London)*, **48**, x (1914)
42. Hill, A. V., *Ergeb. Physiol.*, **15**, 340-479 (1916)
43. Hill, A. V., *Brit. Med. Bull.*, **12**, 165-66 (1956)
44. Meyerhof, O., *Arch. ges. Physiol.*, **182**, 232-83, 284-317 (1920)
45. Parnas, J., *J. Physiol. (London)*, **49**, vii-viii (1914)
46. Parnas, J., *Zentr. Physiol.*, **30**, 1-18 (1915)
47. Parnas, J., and Wagner, R., *Biochem. Z.*, **61**, 387-427 (1914)
48. Peters, R. A., *J. Physiol. (London)*, **47**, 243-71 (1913)
49. Rolleston, H. D., *J. Physiol. (London)*, **11**, 208-25 (1890)
50. Sanderson, J., *Schäfer's Text Book of Physiology*, **2**, 397-407 (Pentland, Edinburgh, Scotland, and London, Engl., 1900)
51. Schiff, M., *Arch. Physiol. norm. path.*, **2**, 157-78 (1869)
52. Stewart, G. N., *J. Physiol. (London)*, **12**, 409-25 (1891)
53. Valentin, G., *Virchow's Arch. pathol. Anat. u. Physiol.*, **28**, 1-29 (1863)
54. Verzár, F., *J. Physiol. (London)*, **44**, 243-58 (1912)
55. Weizsäcker, V., *Arch. ges. Physiol.*, **141**, 457-78; **147**, 135-52; **148**, 535-63 (1911-12)
56. Weizsäcker, V., *J. Physiol. (London)*, **48**, 396-427 (1914)
57. Weizsäcker, V., *S. B. Heidelberg Akad. Wiss.*, 1-63 (1917)

TRANSPORT THROUGH BIOLOGICAL MEMBRANES¹

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The following topics are omitted from this review: transport across plant membranes, capillary permeability, and participation of ion transfer in cardiac activity. Two symposia of general interest were published: *Metabolic Aspects of Transport Across Cell Membranes* (200) and *Hormonal Control of Water and Salt-Electrolyte Metabolism in Vertebrates* (33). Professor F. G. Donnan, whose theory of ionic equilibria across semipermeable membranes is indispensable to physiologists, died in December, 1956, at the age of 86.

GENERAL ASPECTS OF TRANSFER THROUGH MEMBRANES

Pumps and carriers.—Research has shifted from attempts to establish whether a given transfer is active or passive to an effort to understand the intimate nature of active transport [see, however, Jardetzky (141)]. Mitchell (197) proposed that group-transferring enzymes (called translocases) located in the membrane transfer materials by means of covalently linked groups (translocators):

Metabolic energy is generally converted to osmotic work by the formation and opening of covalent links between translocators in the membrane and the carried molecules exactly as in enzyme-catalyzed group-transfer reactions (197).

In a stimulating brief review, Burgen (30) accepted the lipid-core sandwich membrane with inner and outer protein layers stabilized by interconnecting peptide chains:

If the opposing protein laminae lining a pore had an array of groups . . . such that the ion formed a stable combination of two protein groupings, then this arrangement will be specific for ions of particular radius and charge (30).

He thinks that unidirectional movement might occur along a free-energy gradient achieved by a conical matrix. Detachment of the ion at the face of the membrane might be accomplished by mechanical contraction and expansion of the matrix. Adenosinetriphosphate could then serve as a common energy source for many kinds of active transport (80). Transport by this type of mechanism might be highly efficient and does not demand a fixed ratio between the number of ions transported and molecules of adenosinetriphosphate split, since the number of filled pores may vary.

Leake & Pomerat (173) wrote that pinocytosis ("cell drinking") may be concerned in active transport. As originally described, pinocytosis is too slow to account for experimental observations [Christensen *et al.* (35); Gosselin (102)]. Furthermore, it does not accomplish selective concentration of extracellular solute. Perhaps outwardly directed active water transport

¹ The survey of literature pertaining to this review was concluded in May, 1958.

coupled with pinocytosis could result in cell concentrations exceeding those of the medium; this is, however, an unnecessary complication. Hypotheses, having in common the attachment of ions or particles to the surface of the membrane by hydrogen bonding or selective binding, were suggested by Bennett (15). Particles may be carried along into a sink by a moving membrane or may be trapped in vesicles. Enzymatic destruction of the engulfed membrane may follow. It is difficult to see how this type of process can account for many well-established experimental findings (e.g., a linked sodium-potassium transport or the kinetics of cation transfer).

Attempts were made to show that cell fractions or materials isolated from tissues had a selective affinity for sodium or potassium. Such a material might be considered a potential cation "carrier", or, in the words of Baird *et al.* (8), "No generally accepted picture has yet been developed for the mechanism of this hypothetical activity of the membrane, and so it seems justifiable to consider alternative possibilities." One of them is that intracellular structures show a preference for potassium and that no carrier is involved. Szent-Györgyi (267) stated that potassium does not disturb the crystalline regularity of the water structure of cells, while sodium does. Baird *et al.* (8) reported that glycerinated muscle, under certain conditions, temporarily achieved a K/Na ratio of up to 1.8, but that this soon fell and could not be maintained.

Most workers, however, have searched for a possible carrier in the cell membrane. The properties of the hypothetical carrier may be tentatively established: it should be lipid soluble, should bind cations, and may prefer potassium to sodium. Solomon, Lionetti & Curran (263) isolated a heterogeneous material from human red blood cells which bound cations and favored potassium in preference to sodium. This material was soluble in lipid solvents and dissociated in water. They found that some purified lipides, such as phosphatidyl-serine, sphingomyelin, and acetal phosphatide, also combined with potassium in preference to sodium.

Kirschner (158) isolated phosphatidyl-serine which contained sodium in excess of potassium from a radioactive phospholipide prepared by incubating hog red blood cells with Na^{24} . Since the red blood cells of both hogs and men have high $[\text{K}]_i$, these conflicting results may be attributed to differences in isolation procedures. An attempt to show the selective affinity of a number of nucleotides for sodium or potassium was not successful (279).

The fact that cardiac glycosides inhibit cation transport in erythrocytes in extremely low concentrations, by competing with potassium for the carrier, permitted estimation of the number of carrier sites per erythrocyte for which the drug and potassium compete. Glynn (97) estimated about 1000 carrier sites per cell, while Solomon *et al.* (262) estimated about 12,000. In either case the number is remarkably small. If the number of carrier sites bears any relationship to the amount of carrier, which seems reasonable, then the task of isolating such small amounts from the red cell mass is a formidable one. Conway & Duggan (45) estimated that yeast contains 0.12 m.eq. per kg. general cation carrier.

Ling's "fixed charge" hypothesis has been denounced [Conway (41, 42)] and upheld [Ling (175)]. It has reappeared, in somewhat altered form, from Australia [Simon *et al.* (259)]. The hypothesis advanced by Shaw, Simon, and co-workers differs from that of Ling principally in assigning secondary significance to ion radius. They consider potassium to be adsorbed on a charged, semirigid, ordered lattice occupying two-thirds of the cellular volume, while sodium, chloride, and potassium together occupy a third space which is in equilibrium with the external solution. They state that a sodium pump is unnecessary. Harris (113) proposed that in frog muscle the movement of potassium is limited both by a "resistive outer layer" and by diffusion within the cell. The surface resistance could be accounted for as membrane resistance plus the resistance of "an annulus sufficiently thick (ca. 3μ) to accommodate the cell Na". He believes that internal potassium is held on anionic adsorption sites and that an ion can move to a new site only when space is made for it; intracellular diffusion of this ion is slow compared to the rate of passage through the membrane.

Linkage of cation transfer.—Hodgkin (126) discussed linkage of sodium and potassium transport across the squid axon. Proof of a tight coupling is difficult to obtain, since it has thus far been impossible to make certain that $[K]_o$ immediately outside the membrane is actually zero. Evidence indicates that there is probably a loose or partial coupling rather than a tight one-to-one coupling in these giant fibers. In erythrocytes, Glynn (96) showed that although sodium outflux fell when potassium was removed from the external medium, it did not fall to zero. Post & Jolly (219) found, in human red cells, that two atoms of potassium were actively transported inward for every three atoms of sodium actively transported outward. In rat muscle, McLennan (185) proposed that two atoms of sodium are exchanged per atom of potassium.

Water transfer.—The concept of a membrane as a barrier punctured by water-filled pores has been extended to several new tissues. According to Ussing (282) the relative rates of water movement across a membrane by diffusion and osmotic flow depend upon the nature of the barrier [cf. Mauro (196)]. The presence of water-filled pores is indicated by osmotic water flow which is greater than diffusion. From the two measurements one may calculate the equivalent radius of the pores and the proportion of the membrane occupied by pores (fraction pore area) [cf. (229)]. Determinations of this type have been made on frog gastric mucosa (70), mouse stomach (206), gallbladder (106), human erythrocytes (210, 258), and invertebrate nerves (201). The water diffusion permeability of the human red cell was extremely high, the permeability constant calculated being 5.3×10^{-3} cm. per sec. (210). In all instances the fractional pore area was found to be only a very small fraction (less than 1 per cent in some cases) of the total area of the membrane.

Andersen & Ussing (4) used double-labeled thiourea and acetamide to determine the effect of induced osmotic water flow on solute permeability coefficients in the toad skin. These results confirmed the hypothesis that

increased flow through pores exerts a drag force on solute and water so that molecules moving with the stream are speeded up and those moving against it are slowed. Neurohypophyseal hormones increased the pore size. Ussing (283) stated that "since the permeability coefficients can be considered as rate-constants . . . it is readily seen that the solvent drag exerted upon the test substance must result in a drag potential."

Cole & Meredith (38) described a model renal tubule with the general properties attributed to frog skin. Sodium reabsorption was assumed to be an active process and water transfer to be dependent upon the osmotic and hydrostatic pressures across a membrane whose water permeability was controlled by antidiuretic hormone. They showed that for such a membrane the sodium flux is a function of the water flux and vice versa [see also Berliner *et al.* (20)].

The rat ileum was freely permeable to water, which moved in accordance with the osmotic gradient (49); the solution absorbed was isotonic (181). Water reabsorption by the rat jejunum *in vitro* required glucose on the mucosal side (174). Net water outflux from the gallbladder was inversely proportional to $[\text{NaCl}]$ and was calculated to be zero at 220 m.eq. per l. $[\text{Na}]$ (106). The uptake of water from the lumen of the bladder of the intact toad was increased by dehydration and neurohypophyseal hormones (247). The results are in accord with the hypothesis that the hormones open pores in the membrane. However, increased water uptake produced by neurohypophyseal hormones in intact frogs was completely abolished at 1°C. and by anoxia, cyanide, and iodoacetate (133); this suggests an active process. Might the active process be the opening of pores? Pitressin produced maximal water uptake in toads from a 51 mM solution (17).

Metabolism and active transport.—The relationship between transport and oxygen consumption has been of great interest because this comparison may establish or eliminate certain reactions as energy sources for active transport. The many schemes based upon oxidation-reduction reactions have in common that basically a maximum of four cations could be transported per molecule of oxygen consumed ($\text{Na}/\text{O}_2 = 4$) [see Conway (40)]. A number of schemes have been proposed to account for Na/O_2 ratios greater than 4. The whole problem in relation to hydrochloric acid secretion has been discussed by Davies (58) and Davenport (56). There is no agreement as yet.

The frog skin is a particularly attractive preparation for comparisons of this type because active transport of sodium is equivalent to the current which may be drawn from the short-circuited skin. Zerahn (291) determined oxygen consumption by chemical analysis of the solution, while Leaf & Renshaw (171) determined it polarographically. In the latter series of experiments only five out of 120 periods studied gave $\text{Na}/\text{O}_2 < 4$; the mean equalled 6.82.

Since some of the oxygen consumed by the skin must be used by cells other than those transporting sodium, the extra oxygen consumed when this

ion is actively transported is a more informative figure. Zerahn estimated this by determining the oxygen consumption in the absence of sodium transport (Na-free Ringer on the outside). Active sodium transport (obtained by adding sodium outside) always produced a higher rate of oxygen consumption. It was shown that at least eight to ten atoms of sodium could be transported per molecule of oxygen consumed. Leaf & Renshaw (171) determined the increment of oxygen consumption related to the increment of sodium transport produced by the addition of neurohypophysial hormones to the skin. The ratio $\Delta\text{Na}/\Delta\text{O}_2 = 18$. Although use of a ratio of this kind may be criticized, it is clear that sodium is transported in excess of four atoms per molecule of oxygen consumed [cf. (266)].

Zerahn and Leaf & Renshaw made estimates of the possible contribution of anaerobic metabolism to sodium transport. Based upon the current obtained from the skin under anaerobic conditions, about 20 per cent of the energy required for active sodium transport could be contributed by anaerobic metabolism according to Leaf & Renshaw (172).

Cardiac glycosides and inhibition of ion transport.—Glynn (97) determined the effect of digoxin and scillaren A on the components of cation fluxes in human erythrocytes. He wished to separate the possible effect of the glycosides on the metabolic machinery of the cell (in which case only the active fluxes would be inhibited) from their effect on the carrier mechanism itself (in which case other fluxes as well would be inhibited). The results showed clearly that these glycosides affected not only the active fluxes but the passive ones as well. For example, digoxin reduced "downhill" fluxes of both cations: potassium outflux and sodium influx. He concluded that these glycosides inhibited ion transfer in erythrocytes by a mechanism other than preventing metabolic energy from being utilized by the pumps.

Maximum activity of glycosides and related compounds on ion transport in red cells requires the proper combination of a lactone ring with steroid nucleus (147). Potency varies from about 10^{-8} M for ouabain or scillaren A to 10^{-2} M for some simple lactones (97, 147, 262). Inhibition of active cation transport in human red cells by the potent members of this group of substances was not accompanied by loss of high-energy phosphates from the cells [Whittam (286); Kunz & Sulser (165)]. The cardiac glycosides and K appear to compete for a carrier on the cell surface (97, 262). In frog muscle, loaded with Na^{24} by soaking in K-free medium at low temperature, ouabain inhibited the exchange of Na_i for K_o which normally occurs upon incubation in potassium-containing solutions [Johnson (143); Edwards & Harris (72)]. Strophanthin decreased potassium entry and increased its output from K^{42} -loaded frog muscle [Harris (113)].

Much evidence indicates that the cardiac glycosides affect active components of cation fluxes in the heart [Vick & Kahn (285)]. Schreiber (251) found that ouabain produced loss of K_i and gain of Na_i in the isolated beating frog heart. The net loss of K was the result of slowed K influx; no effect

on K outflux was found. In dogs acetylthiocholine produced net loss of potassium and gain of sodium in the myocardium [Regan *et al.* (225)]. Conn's evidence (39) indicates that such an effect, produced in his experiments by chronic administration of digoxin, resulted from decreased rate of entry of potassium into cells. In man, Gollub *et al.* (100) found no significant alteration of the arterial-coronary sinus differences of $[Na]$ and $[K]$ after lanatoside C.

Ion transport in relation to potential difference.—The extensive review by Shanes (253) entitled *Electro-chemical Aspects of Physiological and Pharmacological Action in Excitable Cells* makes it unnecessary to review in detail the relationships between transport and electrical phenomena. The review in this volume on nerve should also be consulted. Hodgkin (126), in a Croonian lecture, reviewed his work on ionic movements and electrical activity in giant nerve fibers.

Adrian (2) found that glass microelectrodes filled with 3 M KCl frequently had tip junction potentials large enough to introduce significant errors into the measurement of membrane potentials. In general, membrane potential depended upon $[K]_o$ and $[K]_i$ at both high and low concentrations. However, at physiological $[K]_o$ the resting potential was about 10 mv. below that calculated from the Nernst equation. Adrian suggested that this must indicate a bound fraction of K_i or that there is some active K uptake. The resting potential of skeletal muscle fibers in perfused limbs depended upon $[K]_o$ when this was altered in the perfusate [Pillat *et al.* (218)].

A view at variance with that held by most authors has been expressed by Shaw, Simon & Johnstone (254). They found no correlation between $[Na]_i$ and overshoot, nor between $[K]_i$ and resting potential when both were determined in the same muscle of *Bufo marinus*. Their work may be criticized for several reasons. Hodgkin (126) pointed out that comparison of $[Na]_i$ with overshoot requires measurement before sufficient water has penetrated to dilute the $[Na]_i$. One may calculate from the data given by Shaw *et al.* that in fresh muscle the sum of $[Na]_i + [K]$ varied from 71 to 130 m.eq. per l. without apparent correlation with water content. Their determination of $[Na]_i$ is uncertain because a constant extracellular volume was assumed. In a subsequent paper, Shaw *et al.* (255) found that $[K]_i$ was not influenced by soaking toad muscle in solutions of varying $[K]_o$ up to 50 m.eq. per l. and that the cell maintained a fairly constant $[Na]_o/[Na]_i$ over a wide range of $[Na]_o$. The result obtained by Shaw and his co-workers are opposed to the usual view that bioelectric potentials in muscle can be calculated from ionic ratios by the Nernst equation or by other simple physicochemical relationships.

Theory.—Robertson (235) reviewed the theory and use of tracers in determining transfer rates in biological systems and Robertson, Tosteson & Gamble (236) analyzed the behavior of tracers in three-compartment closed systems. Kedem & Katchalsky (155) derived equations for permeability of membranes to nonelectrolytes based upon the thermodynamics of irreversible

processes. Petit (216) reviewed osmotic effects in biological systems with emphasis on theoretical aspects.

Membranes.—A theory of dynamic polyionic potentials across ion exchange membranes and verification of the theory have been published by Dray & Sollner (67, 68). Grim & Sollner (107) discussed anomalous osmosis across artificial membranes.

Methods.—An excellent new review on flame photometry was published [Margoshes & Vallee (192)]. Schoffeniels (250) described a new preparation for study of ion flux: the modified isolated single electroplax of the electric eel.

REGULATION OF ELECTROLYTE COMPOSITION OF MAMMALIAN TISSUES AND SECRETIONS

Electrolyte composition of tissues.—Darrow & Hellerstein (55) reviewed factors determining water, sodium, potassium, and chloride composition of the body. The meager data on total body electrolytes obtained by chemical analyses of the human body have now been extended with data for two adult males [Forbes & Lewis (83)]. The results for water were in good agreement with those obtained by the isotope dilution method, but sodium, chloride, and potassium were higher. The discrepancy was greatest for sodium.

Careful determinations of water, fat, and electrolytes in normal muscle samples from patients have been made by Barnes, Gordon & Cope (11), and by Nichols (202). Electrolyte analyses of human gastric mucosa showed that the pattern resembles that previously found in the dog rather than that of the rat and rabbit (194).

Rat thigh muscle had a higher [Cl] and a lower [K] than back muscle, the result of more connective tissue in the former (131). Total extracellular Cl in the rat was estimated by subtracting "nonextracellular Cl" from the total (32). Similar determinations were made for sodium. A 220 gm. rat was calculated to have extracellular fluid volume of 47.4 ml. (from Cl determinations) or 47.9 ml. (from Na determinations).

Comparison of muscle from control and denervated legs of growing puppies showed that denervation produced no change in the concentration of intracellular electrolytes. The denervated muscles had more than normal amounts of fat, but on a fat-free basis a kilogram of denervated muscle contained a larger extracellular phase and a smaller intracellular phase than controls [Eichelberger, Akeson & Roma (74)]. The same changes were found after immobilization of one hind leg (75). Diets deficient in vitamin B₆ were reported to reduce muscle [Na] and increase muscle [K] (134). Muscle biopsies from patients with cirrhosis of the liver showed large chloride spaces without significant alteration in cell water (268).

Differences among tissues in response to withdrawal or addition of extracellular cation were studied (66, 288). Effects of aldosterone on tissue electrolytes were compared with those of deoxycorticosterone; [Na]_i was decreased in brain and muscle and [K]_i was increased in both tissues in spite of an in-

creased plasma $[Na]$ (289). Inhalation of 50 per cent carbon dioxide decreased brain excitability and decreased $[Na+K]_i$ in the brain. Seizures associated with abrupt withdrawal of the 50 per cent carbon dioxide were accompanied by rapid increase in $[Na]_i$ (290).

In the human forearm, uptake of potassium by the muscle followed administration of glucose, potassium chloride, and epinephrine, while loss of potassium accompanied exercise or insulin (110). During the late morning hours, but not at night, there was a net movement of potassium out of the forearm muscles (5). These findings are related to muscle $[K]$ changes in patients with familial periodic paralysis who showed a large net uptake of potassium by the forearm muscles during a nocturnal attack (296) and an abnormally high potassium uptake in response to glucose, epinephrine, and even insulin (109).

The loss of cell potassium and gain of cell sodium commonly found during exposure of isolated tissues to low temperatures may possibly be involved in survival of hypothermic animals. Taylor (272) found that the critical temperature for net loss of potassium from rat diaphragm *in vitro* was 15° to 17°C., while it was significantly lower for the hamster diaphragm. The perfused rat heart, however, did not lose potassium at temperatures as low as 8°C. (272), nor did the perfused cat hind limb at 3°C. (230). Dogs equilibrated with K^{42} , Na^{24} , and Br^{82} before hypothermia lost potassium and sodium from the muscle and potassium from the atria during hypothermia, according to Gollan, Rudolph & Olsen (99).

Artery walls from hypertensive rats contained slightly more water, sodium, potassium, chloride, and phosphorus per unit solids than from controls or normotensive rats (275, 277, 278). Increase in both sodium and chloride suggests an increased extracellular space, and an increase in potassium suggests an increase in size of the intracellular compartment. Daniel & Dawkins (53), however, did not find significant changes from normotensive controls in the potassium or sodium content of aortas from hypertensive rats. Norepinephrine infusion resulted in loss of potassium from the aorta of rats (54) and the femoral artery of dogs (276).

The extracellular space is unexpectedly large in the atria of rabbits (224) and toads (145) when measured with inulin, and probably also in dogs (198). In dogs with experimental chronic heart failure, the ventricular extracellular phase was enlarged but $[K]_i$ was normal (16).

Immature uteri of rabbits and cats had very high extracellular volumes; inulin space was only 60 per cent of the chloride space, and some sodium appeared to be outside the chloride space (52). Injection of ethinyl estradiol into ovariectomized rats produced increased I^{131} -albumin uptake by the uterus within one hour (150). Four hours after injection of estrogen the sodium, chloride, and water content of the uterus reached a maximum; the increase in sodium was greater than could be accounted for by addition of plasma ultrafiltrate (149). Human uterine muscle at the termination of pregnancy contained lower $[K]$ and higher $[Na]$ than nonpregnant uterus (123). Human

endometrium had higher [Na] and [Cl] during the proliferative phase than during the secretory phase (144).

A significant increase in brain [Na] occurred after intact guinea pigs were exposed to 6.6 atm. oxygen (152). The [Cl] in guinea pig cerebral cortex slices was found to vary between 35 and 45 μM /per gm. wet weight, the higher concentration being found in the outer areas. This chloride was almost completely lost by immersion for a few minutes in chloride-free medium (273). Posterior pituitary extract was reported to retard loss of potassium and gain of sodium by guinea pig brain *in vitro* (21, 22).

Bone sodium.—The skeleton contains from about 20 per cent, as in the rat [Cheek, West & Golden (32)], to over 40 per cent, as in adult man [Nichols & Nichols (204)], of the total body sodium. Only a small fraction of this is in the extracellular phase of bone, the greater part being in the crystalline lattice. This bone sodium may act as a reservoir or buffer for the extracellular phase sodium, for it is mobilized during acidosis and negative sodium balance (204). Rapid depletion of sodium in dogs by vivodialysis produced loss of 23 per cent of the total body sodium, of which bone mineral contributed 25 per cent (203). Some tracer sodium in rats entered a part of the bone sodium which did not exchange freely with extracellular sodium (199). The amount of exchangeable bone sodium decreased moderately with age in the rat; a considerable decrease in the proportion of exchangeable bone sodium was partially offset by an increase in total sodium content of the bone (84). Sodium in newly formed bone of young rats was decreased by a low sodium diet (82).

Potassium deficiency.—Potassium deficiency results in loss of this element from the intracellular phase of muscle without an equivalent gain in sodium. It has been suggested that H^+ enters cells to make up the cation deficit, and extracellular alkalosis was found to accompany potassium deficiency. Eckel, Norris & Pope (71), however, found that 8 to 40 per cent of the potassium deficit in muscle of potassium-deficient rats was made up by lysine. This was confirmed by Iacobellis, Muntwyler & Dodgen (137), who found that both lysine and arginine increased in muscle of such rats, while aspartic and glutamic acids decreased. However, such changes in amino acids could not be demonstrated in dogs fed potassium-deficient diets (136). Holliday & Segar (130) fed rats a low-potassium diet which was also low in sodium and chloride and found that although intracellular potassium decreased, there was little sodium replacement; no alkalosis developed as it had in some earlier experiments. Thus, it is probable that alkalosis accompanying potassium-deficiency requires the presence of sodium. Focal myocardial necrosis, commonly described in animals on low-potassium diets, was most prominent when the diet was high in sodium chloride (223). Intestinal smooth muscle is much harder to deplete of potassium than skeletal muscle (50, 51).

The well-known renal lesion produced by potassium deficiency in the rat has now been carefully studied. On such diets containing sodium, the first structural alterations in the kidney occur in the collecting tubules and proxi-

mal convolutions. The lesions of the collecting tubules are probably correlated with impairment of the renal concentrating mechanism and this in turn is correlated with the degree of potassium loss from muscle according to Oliver *et al.* (208) and Hollander *et al.* (129).

Comparison of potassium loss with sodium gain on the basis of total cation content of the tissue can often be misleading because of shift of water between the intra- and extracellular phases. Although calculations of cation concentrations in the intracellular water are necessarily uncertain, particularly when based on an extracellular space determined from sodium or chloride analyses, they are the best available means of comparing cation exchanges in muscle during potassium deficiency. Conway (41) now emphasizes that although radioactive sodium may exchange rapidly with the intracellular sodium of potassium-depleted animals (by exchange diffusion), several days are required for the muscle to return to the essentially sodium-free state. The problem of the rate of transfer of sodium across the muscle cell is not yet settled.

Swelling of tissues in vitro.—Estimates of osmotic activity of tissues from the osmolarity of solutions in which the tissue maintains constant weight necessarily make use of arbitrary end points. Tissues may appear to be hypotonic to the medium 10 min. after immersion and hypertonic 20 min. later [Adolph & Richmond (1)]. Conway's conclusion (41) that tissues are approximately isosmotic with plasma has been confirmed by Appelboom (6). Earlier work by the same group [Brodsky *et al.* (27)] had indicated moderate hypertonicity in tissues, but this seems to have been the result of enzymatic activity in tissue extracts.

Robinson (237) reported that when respiration of rat kidney cortex slices was inhibited by the mercurial diuretic mercaptomerin, water was imbibed from the medium even when this consisted mainly of solutions of choline chloride, choline sulfate, or sodium sulfate. Anaerobic incubation of rat kidney slices at 38°C., followed by transfer into aerobic paraffin oil, was stated to result in extrusion of some water (238). Opie (209) reported that subcutaneous administration of large doses of 25 per cent sodium chloride produced increased "osmotic activity" of liver and kidney slices from these rats when estimated by immersion in media of various tonicities for 10 min. The whole problem has been reviewed by Aebi (3). Robinson (238) concluded that the question of a water pump is still unsettled. However, much information has accumulated to indicate that a water pump is extremely unlikely. There is as yet no thermodynamically sound concept describing water movement across cell membranes which is independent of an osmotic gradient. Much of the previous work which appeared to indicate that tissues extruded water from an hypertonic intracellular phase, as long as energy was present to work the water pump, was at least in part done under unfavorable conditions. Tissues may swell when incubated at 38°C. in serum if oxygenation is inadequate (92).

An interpretation of these results which does not necessitate a water

pump was given by Leaf (169). He found that the swelling of rat cerebral cortex, liver slices, and guinea pig kidney cortex slices at 0°C. was the result of entrance into the tissue of a solution isotonic with the medium; some Na_0 also exchanged for K_i . He showed that active sodium extrusion maintained a high $[\text{Na}]_0$ which counterbalanced a high intracellular concentration of diffusible anions. Failure of the sodium pump, permitting penetration of this ion, has, as a necessary consequence, the penetration of Cl^- , the chief diffusible extracellular anion. Water movement follows the increased cellular osmotic pressure. Leaf's results were confirmed by Itoh & Schwartz (138).

The rat diaphragm, in common with other tissues, gained water from the medium when incubated at 0°C. Normal distribution of water was dependent upon aerobic metabolism [Rixon & Stevenson (233)]. The slight intracellular hypertonicity calculated from analyses of intracellular ions did not disappear upon cooling as would have been expected if the hypertonicity were maintained by a water pump. When incubated in hypotonic or hypertonic media, the rat diaphragm maintained about the same gradient between sodium in the medium and that in the tissue, even though $[\text{Na}]_i$ varied [Rixon & Stevenson (234)]. The intracellular swelling in cold isotonic medium was caused primarily by redistribution of sodium.

Deyrup (65) found that rat kidney cortex slices tended to regulate their water content independently of their sodium and potassium levels. Riecker, Zack & Renschler (232) also presented evidence for an independent movement of water and electrolytes in rat liver.

Measurement of electrolyte distribution in brain tissue is difficult because of the extraordinarily great fluid uptake of brain compared to that of other tissues found by Pappius & Elliott (211). Slices of rat cerebral cortex swelled about 40 per cent after one hour in a bicarbonate buffer. Liver swelled 4 per cent and kidney 12 per cent under comparable conditions. When inulin was present it was taken up with the medium during swelling but did not occupy the entire thiocyanate or sucrose space. This "third space" (the part of the extracellular space which can be penetrated by inulin) probably results from damage to nerve and glial cells during slicing. Earlier findings by Krebs and co-workers that brain slices *in vitro* first lose potassium and then partially regain it under favorable conditions have been confirmed by Pappius & Elliott (212). Glutamate increased potassium uptake and also increased the intracellular volume. When rat cerebral cortex slices were studied in sodium-free medium (containing choline, lithium, or trishydroxymethylamino-methane) the ability to maintain high $[\text{K}]$ in the slice was dependent upon sodium in the medium in spite of high rates of oxygen consumption and glycolysis in the other media. When all the sodium in the medium was replaced by potassium, tissue $[\text{K}]$ exceeded the $[\text{K}]$ in the medium [Pappius *et al.* (213)].

Calcium.—The $[\text{Ca}]$ of squid axoplasm is about 0.4 mM per kg. wet weight [Keynes & Lewis (156)]. The method described permitted analysis of less than 1 μg . Ca in biological material. Only about 2 per cent of the calcium

is ionized [Hodgkin & Keynes (127)]. Outflux of calcium from squid nerve (127) and frog muscle (95) did not seem to be by simple diffusion: active extrusion may occur. The time course of loss of tracer calcium from muscle was complex [Harris (114)]. The calcium of Ehrlich ascites tumor cells was found to be completely exchangeable within a few minutes by Thomason & Schofield (274).

Intraocular fluids.—The participation of aqueous humor dynamics in the regulation of intraocular pressure has been reviewed by Langham (167). Maurice (195) studied the movement of Na^{24} into the aqueous humor and blood after injection of it into the vitreous body. Exchange between the aqueous humor and the vitreous body could be explained by free diffusion across the surfaces separating them, and almost free diffusion within the vitreous body itself.

Davson & Luck (61) found steady state $[\text{Na}]$ in the aqueous humor of the monkey to be 0.97 that in plasma water. An earlier lower value for $[\text{Na}]$ in the aqueous humor was apparently in error because of insufficient equilibration. Thus, in every species examined sodium is present in the aqueous humor in excess of that expected if the aqueous humor were a simple ultrafiltrate. In four species tested the ratio $[\text{Na}]$ in the aqueous humor $[\text{Na}]$ in the serum ($R_{\text{AQ/P}}$) was remarkably constant (0.93 to 0.97), while the ratio for $[\text{K}]$ between the two fluids varied from 0.81 to 1.03 [Harris *et al.* (117)].

In human subjects with cataracts the ratio of $[\text{Cl}]$ between anterior chamber aqueous and plasma was 1.08 [Becker (14)]; the bicarbonate ratio ($R_{\text{AQ/P}}$) was 0.83. In the rabbit, bicarbonate excess may be about 35 per cent and the chloride deficit about 7 per cent. Davson & Luck (62) could not demonstrate any slowing of the turnover of Na^{24} in the aqueous humor after injection of acetazoleamide (Diamox). They and others (14, 104, 168) found that acetazoleamide altered the concentration of the aqueous toward that of a simple ultrafiltrate of the plasma. Thus, acetazoleamide increased $R_{\text{AQ/P}}$ of HCO_3 in man and decreased it in the rabbit.

Ascorbic acid, which is apparently secreted into the aqueous humor by the ciliary processes, occurs in the posterior chamber aqueous humor in higher concentration than in the anterior chamber (12). The secretory process has a maximum rate. The $R_{\text{AQ/P}}$ of ascorbic acid was found to be 15 in man (14). In guinea pigs on a scorbutic diet, the concentration of ascorbate in the anterior chamber aqueous humor decreased without any alteration in the $[\text{HCO}_3]$. Bicarbonate transfer thus does not seem to depend upon ascorbate secretion (13).

Cerebrospinal fluid and blood-brain barrier.—Monographs by Bakay (9) and Herlin (122) on the blood-brain barrier and one by Davson (59) on ocular and cerebrospinal fluids are useful. In nine species of animals the ratio bicarbonate in cerebrospinal fluid/bicarbonate in plasma water was less than one [Davson & Luck (60)]. Acetazoleamide reduced the rate of turnover of sodium in the cerebrospinal fluid by about 40 per cent. The same drug produced a fall in $[\text{Cl}]$ in the cerebrospinal fluid toward that expected from

the Donnan equilibrium. The same trend was found for the bicarbonate ratio [Davson & Luck (62)].

After intravenous injection of K^{42} into the dog, the concentration of the tracer in cerebrospinal fluid reached a maximum in about 30 min., but 2 hr. were required for equilibrium of K^{42} between the cerebrospinal fluid and the brain tissue [Rudolph & Olsen (244)]. Hypoxia increased the rate of passage of radioactive iodinated human serum albumin from plasma to the cerebrospinal fluid in dogs [Slobody *et al.* (260)].

After injection of Cl^{36} , equilibrium between blood and brain chloride required increasingly more time with increasing age, and this trend was not found in liver and muscle (166). No blood-brain barrier could be demonstrated to thiopental in dogs (193). Various properties of the blood-brain barrier determined by means of Br^{82} are described (221).

Placenta.—Garby (89) studied the permeability properties of the isolated human amniotic membrane; molecules as large as albumin penetrated, but most pores were of the order of 5 to 20 Å. He discussed the formation of the amniotic fluid.

The relative importance of the fetus-placenta complex and the fetal membranes in the exchange of water between the maternal circulation and the amniotic fluid was evaluated in the rabbit by simultaneous determination of the rate of passage of DHO from the maternal system to the amniotic fluid and of THO in the opposite direction. Ligation of the vitelline vessels produced no significant reduction in transfer rate, but crushing the fetus halved the rate in both directions [Paul *et al.* (215)]. The rabbit placenta passed the aldehydic forms of hexoses (glucose, galactose, mannose) much more readily than the ketonic forms (fructose, sorbose), according to Davies (57). Glucose inhibited the transfer of sorbose from the mother to the fetus in the rabbit (37). In man, as in the sheep and guinea pig, the transfer of fructose from mother to fetus is much slower than that of glucose or xylose (132). Tracer sodium transfer from mother to fetus could be determined by placing a counter externally over the anterior aspect of the pregnant uterus (36). Tracer phosphate transfer across the placenta has been studied (87). The placenta of the guinea pig and rabbit (112) acted as a barrier to the transfer of thyroxine (125) and of tri-iodothyronine (180), but not of iodide (177).

Gastric secretion.—The subject of gastric acid secretion will not be reviewed here, since in addition to the chapter on digestion in this volume a number of excellent reviews have appeared recently [Öbrink (207); Davies (58); Davenport (56); Rehm & Dennis (226)].

Salivary secretion.—The review by Lundberg (179) includes references to most of the important recent work on electrolyte secretion. Study of the passive secretion of ten nonelectrolytes by the parotid gland of the dog showed that two types of relationship between concentration in saliva and rate of secretion may exist. Analysis of the data suggests that these substances usually penetrate the lipoid phase of the membrane, but that as rate of secretion rises, penetration through water-filled pores may occur [Borgen

(29)]. Both radioactive iodide and thiocyanate were concentrated (i.e., the concentration in the gland exceeded that in plasma) in the submaxillary glands and saliva of hamsters and mice, but not in those of rats nor in the sublingual or parotid glands of any of the three species [Logothetopoulos & Myant (176)].

ELECTROLYTE EXCHANGE IN TISSUES AND ORGANISMS

Erythrocytes.—Red blood cells with four distinct electrolyte patterns can be distinguished in sheep [Evans (77)]. The types of erythrocytes range from the high K—low Na cell characteristic of man to the low K—high Na cell characteristic of the dog and cat. A given animal does not change from one type to another. Recent evidence shows that these differences in red blood cell types are not confined to sheep. Two distinct types of red blood cells have been recognized among goats (78) and Australian opossums (*Trichosurus vulpecula*) (10).

An excellent review of ionic permeability of red blood cell membranes has been written by Glynn (98). Phosphate transfer into chicken erythrocytes appears to derive energy from either glycolysis or respiration [Gourley (103)]. Human red cells depleted of potassium by cold storage and fresh cells differ in their response to purine ribosides; the ribosides inhibit P^{32} uptake in the potassium depleted cells and increase it in the fresh cells [Kahn (146)]. Incorporation of phosphate into organic phosphate will not be considered here [cf. (93)].

Tosteson & Johnson (280) have shown that inhibitors may dissociate glycolysis from potassium influx in duck red blood cells incubated anaerobically with glucose. Duck cells stored anaerobically at 37°C. in the absence of glucose lost their ability to glycolyze and to accumulate potassium. Both these properties were restored by glucose plus adenosine (281). Tortoise erythrocytes utilized energy from respiration for active sodium transport and showed a high requirement for calcium; in the absence of calcium, sodium influx increased [Maizels (187)].

Harris & Pranker (115) studied the kinetics of the uptake and output of radioactive sodium and the uptake of radioactive potassium by human red blood cells. The data were analyzed in terms of the hypothesis that movement of these ions is restricted by slow intracellular diffusion as well as by the cell membrane (113). They consider the red blood cell to have a thin, sodium-rich outer region surrounding a potassium-rich inner region in which potassium is concentrated by selective adsorption.

The extensive experimental results obtained by Glynn (96) have provided us with a much more satisfactory picture of electrolyte penetration into the red blood cell than was hitherto available. Contrary to some previous results obtained by others, Glynn found that potassium influx in human red cells increased with increasing $[K]_0$ in a manner describable by Shaw's equation (256) for horse erythrocytes. This equation contains two components: one of them, the "Michaelis" compartment, is dependent upon glucose and becomes saturated at about 10 mM $[K]_0$; the other, a smaller one, the linear com-

ponent, is proportional to the $[K]_o$. Although sodium outflux was independent of $[K]_o$ at values above 10 mM, it fell by one-third at the lowest $[K]_o$ obtainable. The sodium outflux remaining at $[K]_o = 0$ was not affected by glucose deprivation. Sodium outflux is thus also considered to consist of two components, an active one dependent upon energy derived from glucose and linked to potassium influx, and a passive one unaffected by glucose and persisting in the absence of external potassium (96).

Measurements have been made of ATP, ADP, and 2,3-diphosphoglyceric acid in human red cells under varying conditions of potassium exchange. The data substantiate the idea that ATP is a link between glycolysis and active potassium transport (91,286). Adenosine and inosine augmented the rate of potassium accumulation in red cells depleted of this ion (148).

It now appears unlikely that cholinesterase is involved in cation transport in human red blood cells (105, 128). The rate of sodium extrusion and potassium uptake by red cells was reduced by quinidine (153). Human red cells lost potassium and gained sodium in bicarbonate buffer containing deoxycorticosterone glycoside. The concentration of 2,3-diphosphoglycerate increased in the hormone-treated cells. This is probably a nonspecific effect (257).

Muscle.—McLennan (182, 184) reported that the diffusion constant of potassium in the extracellular space of muscle and brain, but not liver, was only about 7 per cent that in free solution; later he (186) showed that in the extracellular space of rat diaphragm it was approximately that in free solution, provided the solution used for suspension of the diaphragm was Ringer's. The earlier result obtained in potassium phosphate solution was attributed to the effect of phosphate on the diffusion of potassium.

In rat muscle a proportion of potassium which is exchangeable at 20° became nonexchangeable at 0°C. [McLennan (183)]. Metabolic depression (cooling, dinitrophenol, azide, cyanide) caused a large decrease in the total muscle potassium of extensor digitorum longus muscles of the rat mainly because of a decrease in potassium influx (183). Using the same muscle, McLennan (185) found that all the sodium was exchangeable with that of saline medium at 20°C. Loss of labeled sodium from the loaded muscle, after rapid loss from the extracellular space, occurred with a uniform time constant. There was no evidence of a difficulty exchangeable fraction as had been found in frog muscle. Harris & Steinbach (116) studied the relative specific activity of tracer sodium and potassium in water or sugar solution extracts of frog muscle. A fraction of the sodium which could not be leached out was associated with the ends of the muscle, suggesting that it was present in connective tissue. There was a nonuniform exchange of muscle potassium with tracer potassium. Output of radioactive sodium from loaded frog muscle was reduced by lowering the temperature from 20° to 0°C., by omitting potassium from the medium or by ouabain [Edwards & Harris (72)]. They proposed that "most of the Na turnover measured with tracers takes place between an outer depot and the solution."

Conway & Carey (44) found that the $[Na]_i$ of frog muscle was increased

by soaking overnight at 0°C. in potassium-free solution containing 120 m.eq. per l. Na and then immersing the muscle for 2 hr. at room temperature in fluid containing 104 m.eq. per l. Na and 10 m.eq. per l. K. The added Na_i was extruded by an active process and this extrusion was inhibited by cyanide and anoxia, and to some extent by dinitrophenol [Conway (41, 42)].

Rat diaphragm incubated *in vitro* below pH 7 lost more sodium and potassium than at pH 7.4. At the lower pH intracellular K^+ exchanged for extracellular H^+ [Rogers (239)]. Cholinesterase inhibitors are claimed to inhibit the sodium pump in frog muscle [Van der Kloot (284)].

Briner, Simon & Shaw (26) studied inorganic phosphate uptake by the sartorius muscle of *Bufo marinus* and found that increases in extracellular inorganic phosphate concentration increased muscle phosphate, but that the inorganic phosphate space was smaller than the chloride space. On the basis of the hypothesis proposed by Simon & Shaw for a three-compartment system in toad muscle, they interpret their results to mean either that phosphate does not penetrate all of the "free intracellular phase" (the third compartment) or that chloride penetrates the ordered phase of this muscle. Both phosphate and chloride spaces exceeded the inulin space. Manery, Gourley & Fisher (191) found that insulin and, more strikingly, insulin plus lactate caused uptake of potassium by frog muscle. Oxygen consumption was also stimulated.

Muscle: abnormal cations and anions.—Replacement of part of the chloride by sulfate in fluid perfusing cat hind limbs caused release of potassium, reversible contracture, and decrease in muscle resting potential, probably the result of increased permeability to sodium [Giebisch *et al.* (94)].

In frog muscle, substitution of NO_3 or Br for all Cl in the medium, or substitution of I for part of the Cl, had no immediate effect on Na turnover or K uptake. Loss of radioactive K from the muscle was increased by I; SCN inhibited K uptake [Edwards, Harris & Nishie (73)].

Relman (227) reviewed the physiological behavior of Rb and Cs in relation to K. More than half the muscle K of intact rats may be replaced by Rb or Cs [Relman *et al.* (228)]. The K of frog sartorius muscle could be replaced by Rb or Cs with little change in Na by soaking in solutions containing 10 mM per l. of the abnormal cation instead of K [Lubin & Schneider (178)]. The interrelationships among K, Rb, and Cs in muscle are complex and these cations cannot be easily arranged in an "immutable sequence".

Kidney.—Only exchange in renal tissue *in vitro* will be considered here. The chapter on kidney should be consulted for further information. Trans-tubular electrical potential differences must now be considered in interpretation of results of electrolyte uptake by the kidney. Solomon (264) found that the lumen was negative to the outside of the kidney and that a bimodal distribution could be established with ranges of 19 to 39 mv. and 34 to 70 mv. The lower potential differences seemed to be those of the proximal tubules.

Cort & Kleinzeller (46) compared electrolyte exchange in slices from

normal and denervated rabbit kidneys. Two weeks after denervation, these slices showed a reduced resistance of the membrane to passive sodium influx and a reduced active sodium extrusion. Both potassium and rubidium entered leached kidney cortex slices from normal rabbits in the same way, probably passively (160). Lithium, which entered the slices during leaching, could not be transported outward against a concentration gradient as could sodium (47). Protamine, and to a lesser extent ammonium, polylysine, and imidazole, inhibited potassium uptake by leached kidney cortex slices. The effect appeared to be specific for potassium uptake, since inhibition of this process by protamine also occurred if simultaneous net sodium extrusion was prevented by high extracellular $[Na]$ as shown by Foulkes & Miller (86). Very high pressures of oxygen *in vitro* (11 atm.) produced loss of potassium and gain of sodium in kidney slices (151).

Kleinzeller & Cort (159) determined the effect of some mercurial diuretics on electrolyte and water exchange in slices of rabbit kidney cortex. Slices leached at $0^{\circ}C$. in saline containing the mercurials took up more sodium and water than did controls. Upon incubation at 37° both were lost and potassium was gained at the same rate as in controls. These and other results are interpreted to mean that the mercurials increased passive movement of sodium, chloride, and water in kidney slices without effect on active sodium transport. These results contradict earlier findings by others.

Slices of cortex from several mammalian kidneys accumulate S^{35} *in vitro*, but slices from infant rat kidneys and three species of lower vertebrates failed to accumulate S^{35} [Deyrup (63)]. After accumulation of S^{35} had taken place, such agents as dinitrophenol, Hg^{++} , Ca^{++} , Na^{+} , or anaerobiosis, previously shown to decrease uptake, produced loss of S^{35} from the tissue. However, at $1^{\circ}C$., loss produced by these inhibitors was slight [Deyrup (64)]. Deyrup proposed that two processes are involved in S^{35} accumulation: entrance into the cells, and combination with an intracellular component to form a complex which is stable at low temperature. Differences in sulfate uptake, suggestive of different sulfate compartments, were found when sulfate was determined by chemical and tracer methods [Berglund & Deyrup (19)]. Cadmium¹¹⁵ was accumulated selectively in the cortex of the rat kidney [Gunn & Gould (111)].

Frog skin.—Measurement of potentials with microelectrodes inserted into the frog skin showed that the P.D. developed in two, or, rarely, three distinct jumps rather than in one as reported by others [Engbaek & Hoshiko (76)]. These new findings are in agreement with the hypothesis suggested by Ussing that the P.D. is the sum of a sodium diffusion potential at the outer border, and a potassium diffusion potential at the inner border of the epithelial cells. Evidence for this hypothesis has just appeared (163a). The outside of frog skin behaves as if it were selectively permeable to sodium, while the inside behaves like a potassium electrode. The theory assumes that the sodium pump is located at the inward-facing membrane of the epithelial cells. The pump itself is not considered to be electrogenic, but to keep cell

sodium low through exchange of it against potassium (and possible H^+) of the solution in contact with the inside of the skin.

When unbuffered solutions were used, a H^+ gradient across the frog skin developed very rapidly, then remained constant [Fleming (81)]. Schoffeniels (249) suggested that H^+ and Na^+ compete for the carrier in frog skin. Huf, Doss & Wills (135) suggested that a distinction be made between processes in frog skin related to "unidirectional active ion transport" and processes related to "maintenance electrolyte equilibrium". They found that several metabolic inhibitors, particularly fluoroacetate, decreased active ion transport without affecting the sodium and potassium content of the skin. Most inhibitors decreased both active transport and skin $[K]$. A mechanism of active ion transport based upon an "exchange-adsorption" reaction involving a redox carrier was proposed (135). Snell & Leeman (261) determined the temperature coefficients of sodium transport in frog skin. The Q_{10} for the current was about 2. Net flux of sodium varied 9 to 10 per cent per degree C. The data suggest that transport becomes increasingly efficient at low temperatures. Another scheme for sodium transport in the frog skin was proposed by Kato, Zwolinski & Eyring (154). They assumed that sodium influx occurs chiefly as a complex with a substance in the membrane which facilitates passage over rate limiting barriers; the complex is thought to be formed at one boundary and broken at the other.

Tashiro (269) studied chloride and phosphate transfer across the frog skin. Effects of 5-hydroxytryptamine (217) and adrenal steroids (271) have been reported.

Bladder.—A characteristic potential difference occurs across the isolated urinary bladder of the toad (170, 270). As an experimental membrane the bladder is simpler than the frog skin, since it consists of a single layer of epithelial cells supported on a fine layer of connective tissue. Leaf, Anderson & Page (170) found that aerobically the entire potential difference was the result of active sodium transport. Anaerobically the short-circuit current was frequently well maintained for several hours, and the net sodium flux was about 15 per cent less than the short-circuit current. Pure oxytocin or vasopressin increased the current when applied to the serosal side under either aerobic or anaerobic conditions. Tashiro (270), however, reported chloride influx across the bladder of *Bufo vulgaris Japonicum* to be much greater than that predicted from Ussing's formula.

Subcellular distribution of electrolytes.—In rat liver homogenate about 75 per cent of the total potassium and sodium were found in the supernatant fraction; the rest of the potassium was distributed among nuclei, mitochondria, and microsomes [Berger (18)]. Potassium associated with the subcellular particles was bound in a nondiffusible form (18, 108). Rat liver mitochondria maintained a relatively low water content and an ionic gradient with the medium when adenosine phosphates were present. In the absence of these, mitochondria swelled and their $[Na]$ and $[K]$ decreased (220). This apparently active cation uptake was not observed by Jackson & Pace (140).

The [K] in mitochondria and in the soluble fraction was reported to be altered by hormones and drugs (7). Rat liver mitochondria and their fragments prepared by disruption with digitonin selectively bound more potassium than sodium and retained their ability to catalyze oxidative phosphorylation. Both potassium-binding ability and oxidative phosphorylative catalysis are absent in microsome and mitochondrial fragments prepared by mechanical disruption [Gamble (88)]. Since these new mitochondrial fragments prepared with digitonin lack a membrane, they are a valuable addition to systems in which potassium-binding may be studied. Nuclei isolated from the cat's thymus appear to contain relatively more sodium and less potassium than the cytoplasm (139).

Ascites tumor.—The Ehrlich mouse ascites tumor was used for the study of exchange of sodium and potassium *in vitro*. For this purpose the cells may be handled in much the same manner as erythrocytes with allowance being made for the much larger volume (14 per cent) of trapped intercellular fluid. Maizels, Remington & Truscoe (188) studied penetration of sodium into the tumor cells. Sodium exchange was extremely rapid: between 21 and 42 p.moles per cm.² per sec. In the same terms, exchange in frog muscle is about 5 and in human red cells about 0.06. The same authors (189) found that the Q_{10} of Na influx and outflux varied between about 2 and 4. Sodium and potassium appear to exchange on a one-to-one basis. Cardiac glycosides decreased the rate coefficient of both outflux and influx of sodium. Apparently energy from both respiration and glycolysis may be used for sodium transport in ascites cells (190). Hempling (121) made somewhat similar measurements using both radioactive sodium and potassium. He concluded that both potassium accumulation and sodium extrusion can occur against electrochemical gradients but that the transport of these two ions may be independently varied. For example, at 5°C. fluxes of the two ions were different, and after the cells had been at 2°C. exogenous glucose increased the accumulation of potassium only, without any effect on sodium. Further, the temperature coefficients of sodium and potassium fluxes were markedly different.

Invertebrates.—The wool-handed crab (*Eriocheir sinensis*), which can live in salt or fresh water, can absorb either sodium, potassium, or lithium from an 8 mM solution. Potassium did not interfere with sodium absorption, but lithium decreased the absorption of all cations when present [Koch & Evans (161)]. The same animals (with closed urinary pores), placed in distilled water, showed a net loss of sodium until the outside concentration reached between 0.03 and 0.5 mM where a steady state prevailed. Sodium turnover occurred while the steady state was developing as well as afterward (162). Sodium uptake from dilute sodium solutions was inhibited by thionine. At very low concentrations ($10^{-7}M$) it increased loss of sodium and at higher concentrations decreased uptake (163). Isolated gills from this crab absorb sodium from dilute solutions. This absorption is inhibited by certain antibiotics [Dumont (69)]. The displacement of intracellular potassium by H^+

was reported to occur in the isolated heart of *Helix pomatia* [Stolkowski & Reinberg (265)].

Microorganisms.—Potassium uptake by yeast was separated into an azide-sensitive and an azide-insensitive mechanism. The latter required glucose and phosphate and functioned both aerobically and anaerobically [Foulkes (85)]. The general cation carrier in yeast was reported by Conway & Duggan (45) to transport not only K but also H^+ , Rb, Cs, Na, Mg, and Ca in that order of effectiveness. Magnesium and potassium competed for the carrier at pH 7 [Conway & Beary (43)]. When yeast was provided with sugar (glucose, fructose, or mannose) as substrate, phosphate was actively transported into the cell. Glycolysis furnished the energy. The optimum pH of 6.5 was shifted to about 5 in the presence of K which stimulated phosphate uptake within a restricted pH range [Goodman & Rothstein (101)]. Rothstein & Hayes (242) continued study of cation binding by yeast cell surfaces. Rothstein *et al.* (243) and Jennings *et al.* (142) found that manganese and magnesium were absorbed by yeast cells by an apparently active process which required that phosphate also be absorbed.

Miscellaneous.—Under the stimulus of an osmotic load, cormorants secrete from the beak—probably from the nasal gland—an almost pure sodium chloride solution of about 500 mM per l. [Schmidt-Nielsen *et al.* (248)]. Fluid is produced by rabbit oviducts at a pressure as high as 71 cm. H_2O (23). Diffusion through rabbit ureters was studied (90). Seminal vesicle mucosa of the guinea pig is able to utilize both aerobic and anaerobic energy for maintenance of a cation concentration gradient (25). The ability of the mammary gland to accumulate iodine was studied in detail (28). The [K] of the udder lymph of cows was higher than could be accounted for on the basis of the Donnan equilibrium (124).

EXCHANGE OF SUGARS AND AMINO ACIDS

Sugar transport in relation to insulin action.—Studies of the distribution of sugars in both isolated tissues and intact animals provide further evidence in favor of the hypothesis proposed by Levine and Goldstein that at least one of the actions of insulin is to increase the rate of transfer of glucose into the intracellular space of muscle. Evidence for the hypothesis has been reviewed by Ross (241). The concentration of these sugars inside the cells never exceeds that in the extracellular phase, so that by most definitions it is not an active transport. Further, the effectiveness of insulin in permitting glucose to pass the cell membrane differs from one tissue to another and is greatest in muscle.

A number of authors reported studies on pentose distribution. Since there is no known mechanism by which pentoses are phosphorylated in muscle, distribution of pentose in excess of the extracellular space of muscle indicates penetration into the intracellular phase. Park and co-workers (214) extended their earlier observations with glucose to other sugars and have shown that without insulin the intracellular free-sugar concentration in skeletal muscle

is approximately zero, while with insulin the intracellular free-sugar concentration rises. D-Mannose, D-fructose, D-xylose, and L-arabinose were used in this manner (214). In nephrectomized cats, the pentose space of muscle for L-arabinose and D-xylose was equal to the chloride space in the absence of insulin. In the brain and heart, pentose space exceeded the chloride space. In all three tissues the pentose space was increased by administration of insulin. In other tissues in which the pentose space equalled chloride space (intestines, lung, skin, and spleen), insulin had no effect [Sacks & Bakshy (245)]. The enantiomorphs of these sugars were not subject to insulin control. All these sugars (245) and others (31) distribute freely in the total water of the liver so that the concentration in liver water is equal to that in plasma water. The pentose space of L-arabinose and D-xylose, which was increased by insulin, was also increased by muscular activity, and the two effects were additive. This suggests that the effects are exerted through different mechanisms (246). Galactose penetration into the perfused rat heart was increased by insulin and decreased by glucose. The rate of glucose utilization was decreased by galactose [Fisher & Lindsay (79)]. In nephrectomized rats, Helmreich & Cori (120) found that the distribution of five aldo-pentoses, galactose, and arabinose was increased by insulin, or by muscular activity. This was partly the result of increased utilization and partly the result of increased volume of distribution. Galactose was reported to penetrate the isolated diaphragm in the absence of insulin but the rate of penetration was increased by insulin. The insulin-dependent transfer was inhibited by dinitrophenol and iodoacetic acid [Resnick & Hechter (231)]. Their diaphragm preparation was not entirely suitable because cutting of muscle fibers was necessary for its preparation. Kipnis & Cori (157) described a new rat diaphragm preparation in which cut muscle fibers were avoided and the extracellular space, therefore, kept small. D-xylose penetrated this preparation by diffusion, and insulin increased both the rate of penetration and the volume of distribution of the sugar. In man the injection of insulin resulted in abrupt lowering of the blood level of D-xylose, L-arabinose, and D-lyxose [Segal, Wyngaarden & Foley (252)]. The effect was attributed to increase in the volume of distribution of the sugars.

In Ehrlich ascites tumor cells *in vitro* a number of sugars were found to penetrate the cells in a manner describable by a reversible, first-order process. No concentration of the sugars in the intracellular water in excess of that in the medium occurred and insulin had no effect. However, penetration of a given sugar was strongly temperature dependent (Q_{10} equaled about 4). It was suggested that this rather high Q_{10} was the result of alteration of the properties of the membranes by cooling [Crane, Field & Cori (48)].

The intracellular enzyme, aldolase, is lost from tissue upon incubation [Zierler (292)]. This leakage was increased by anoxia, glucose-lack, and high [K] in the medium and occurred both from diaphragm and from the peroneus longus muscle of the rat (293). In the latter preparation, insulin increased

the rate of loss of aldolase into the medium and also the uptake of glucose. However, similar effects were produced by the addition of roughly equivalent amounts of protein as Cohn's fraction V human plasma albumin (295). The same author reported that the addition of insulin *in vitro* to rat muscle increased the resting potential by from 5 to 8 mv. without sufficient increase in $[K]_i$ to account for it [Zierler (294)]. The process by which glucose is excluded from the interior of the cell when insulin is absent appears to be dependent upon oxidative energy (222). Krahl (164) speculated on a possible action of insulin which can account for its varied effects in tissues. The proposal is that insulin initiates a series of molecular interactions at the extracellular-intracellular boundary, and that these in turn propagate a series of rearrangements such that intracellular barriers are removed between hexokinase and its substrates. This hypothesis attempts to link the permeability hypothesis of insulin action and its effect on enzyme systems of the cell.

Erythrocytes.—Bowyer (24) reviewed the kinetics of penetration of nonelectrolytes into mammalian erythrocytes [see also Wilbrandt (287) for a brief review]. Bowyer concluded that the transfer rate of glucose and glycerol across red blood cell membranes is proportional to the difference between the reciprocals of the concentrations on the two sides. She discussed several model transport schemes which give the required kinetics.

Convincing evidence that glucose transport across the erythrocyte membrane requires a carrier was the demonstration by Rosenberg & Wilbrandt (240) and by Park and co-workers that under proper conditions a "flow-induced uphill transport" can be produced in a system where a carrier has affinity for two substrates. Rosenberg & Wilbrandt did this by adding glucose or mannose to red blood cells previously equilibrated with C^{14} glucose. Inward transport of the sugar brought about temporary outward movement of the C^{14} glucose against a concentration gradient. Park and co-workers used glucose to bring about an outward movement of xylose in an uphill direction. Rosenberg and Wilbrandt state that this type of transport is

a feature characteristic for mobile carrier systems and is not to be expected in systems in which the substrate is bound to a fixed membrane component, although such a system may yield identical transport kinetics.

Amino acids.—Ehrlich mouse ascites tumor cells concentrate glycine against a concentration gradient. Glycine accumulation is an active process; the influx coefficient was reduced by inhibitors without altering the outflux coefficient [Heinz (118)]. In the steady state, less than three molecules of glycine are transported per molecule of oxygen consumed (119). Other amino acids are also concentrated by these cells [Christensen *et al.* (34)]. A nonmetabolizable amino acid, α -aminoisobutyric acid, is concentrated by mammalian cells to several times the plasma level [Noall *et al.* (205)]. The accumulation of this amino acid is under hormonal control. Epinephrine increased the α -aminoisobutyric level in all tissues compared with plasma,

and growth hormone increased the accumulation in all tissues except heart. Hydrocortisone increased the uptake by liver, and estrogen increased the uptake by uterus. This is another instance in which hormonal control appears to operate by altering the availability of a material in the intracellular phase of tissues.

LITERATURE CITED

1. Adolph, E. F., and Richmond, J., *Am. J. Physiol.*, **187**, 437 (1956)
2. Adrian, R. H., *J. Physiol. (London)*, **133**, 631 (1956)
3. Aebi, H., *Deut. med. J.*, **7**, 429 (1956)
4. Andersen, B., and Ussing, H. H., *Acta Physiol. Scand.*, **39**, 228 (1957)
5. Andres, R., Cader, G., Goldman, P., and Zierler, K. L., *J. Clin. Invest.*, **36**, 723 (1957)
6. Appelboom, J. W., *Federation Proc.*, **16**, 278 (1957)
7. Auditore, G. V., and Holland, W. C., *Am. J. Physiol.*, **187**, 57 (1956)
8. Baird, S. L., Jr., Karremann, G., Mueller, H., and Szent-Györgyi, A., *Proc. Natl. Acad. Sci. U. S.*, **43**, 705 (1957)
9. Bakay, L., *The Blood-Brain Barrier* (Charles C Thomas, Publisher, Springfield, Ill., 154 pp., 1956)
10. Barker, J. M., *Nature*, **181**, 492 (1958)
11. Barnes, B. A., Gordon, E. B., and Cope, O., *J. Clin. Invest.*, **36**, 1239 (1957)
12. Becker, B., *Am. J. Ophthalmol.*, **41**, 522 (1956)
13. Becker, B., *Am. J. Ophthalmol.*, **44**, Part II, 402 (1957)
14. Becker, B., *Arch. Ophthalmol. (Chicago)*, **57**, 793 (1957)
15. Bennett, H. S., *J. Biophys. Biochem. Cytol.*, **2**(4), Part 2, Suppl., 99 (1956)
16. Benson, E. S., Freier, E. F., Hallaway, B. E., and Johnson, M. J., *Am. J. Physiol.*, **187**, 483 (1956)
17. Bentley, P. J., *J. Endocrinol. (London)*, **16**, 126 (1957)
18. Berger, M., *Biochim. et Biophys. Acta*, **23**, 504 (1957)
19. Berglund, F., and Deyrup, I., *Am. J. Physiol.*, **187**, 315 (1956)
20. Berliner, R. W., Levinsky, N. G., Davidson, D. G., and Eden, M., *Am. J. Med.*, **24**, 730 (1958)
21. Bernard-Weil, E., *Ann. Endocrinol. (Paris)*, **18**, 445, (1957)
22. Bernard-Weil, E., Guérin, M. T., Guérin, R. A., and Decourt, J., *Compt. rend. soc. biol.*, **151**, 445 (1957)
23. Bishop, D. W., *Am. J. Physiol.*, **187**, 347 (1956)
24. Bowyer, F., *Intern. Rev. Cytol.*, **6**, 469 (1957)
25. Breuer, H. J., and Whittam, R., *J. Physiol. (London)*, **135**, 213 (1957)
26. Briner, G. P., Simon, S. E., and Shaw, F. H., *J. Gen. Physiol.*, **41**, 755 (1958)
27. Brodsky, W. A., Appelboom, J. W., Dennis, W. H., Rehm, W. S., Miley, J. F., and Diamond, I., *J. Gen. Physiol.*, **40**, 183 (1956)
28. Brown-Grant, K., *J. Physiol. (London)*, **135**, 644 (1957)
29. Burgen, A. S. V., *J. Cellular Comp. Physiol.*, **48**, 113 (1956)
30. Burgen, A. S. V., *Can. J. Biochem. and Physiol.*, **35**, 569 (1957)
31. Cahill, G. F., Jr., Ashmore, J., Earle, A. S., and Zottu, S., *Am. J. Physiol.*, **192**, 491 (1958)
32. Cheek, D. B., West, C. D., and Golden, C. C., *J. Clin. Invest.*, **36**, 340 (1957)
33. Chester Jones, I., and Eckstein, P., Eds., *Mem. Soc. Endocrinol.*, No. 5, Part II, pp. 1-124 (1956)

34. Christensen, H. N., Riggs, T. R., Aspen, A. J., and Mothon, S., *Ann. N. Y. Acad. Sci.*, **63**, 983 (1956)
35. Christensen, H. N., Noall, M. W., Riggs, T. R., and Walker, L. M., *Science*, **127**, 163 (1958)
36. Clayton, C. G., Farmer, F. T., and Johnson, T., *Lancet*, **II**, 539 (1956)
37. Colbert, R. M., Calton, F. M., Dinda, R. E., and Davies, J., *Proc. Soc. Exptl. Biol. Med.*, **97**, 867 (1958)
38. Cole, D. F., and Meredith, J. F., *Bull. Math. Biophys.*, **19**, 23 (1957)
39. Conn, H. L., Jr., *Am. J. Physiol.*, **184**, 548 (1956)
40. Conway, E. J., *Intern. Rev. Cytol.*, **4**, 377 (1955)
41. Conway, E. J., *Physiol. Revs.*, **37**, 84 (1957)
42. Conway, E. J., in *Metabolic Aspects of Transport across Cell Membranes*, 73, 179, 186 (Murphy, Q. R., Ed., University of Wisconsin Press, Madison, Wis., 379 pp., 1957)
43. Conway, E. J., and Beary, M. E., *Nature*, **178**, 1044 (1956)
44. Conway, E. J., and Carey, M., *Nature*, **178**, 644 (1956)
45. Conway, E. J., and Duggan, P. F., *Nature*, **178**, 1043 (1956)
46. Cort, J. H., and Kleinzeller, A., *J. Physiol. (London)*, **133**, 287 (1956)
47. Cort, J. H., and Kleinzeller, A., *Biochim. et Biophys. Acta*, **23**, 321 (1957)
48. Crane, R. K., Field, R. A., and Cori, C. F., *J. Biol. Chem.*, **224**, 649 (1957)
49. Curran, P. F., and Solomon, A. K., *J. Gen. Physiol.*, **41**, 143 (1957)
50. Daniel, E. E., and Bass, P., *Am. J. Physiol.*, **187**, 247 (1956)
51. Daniel, E. E., and Bass, P., *Am. J. Physiol.*, **187**, 253 (1956)
52. Daniel, E. E., and Daniel, B. N., *Can. J. Biochem. and Physiol.*, **35**, 1205 (1957)
53. Daniel, E. E., and Dawkins, O., *Am. J. Physiol.*, **190**, 71 (1957)
54. Daniel, E. E., Dawkins, O., and Hunt, J., *Am. J. Physiol.*, **190**, 67 (1957)
55. Darrow, D. C., and Hellerstein, S., *Physiol. Revs.*, **38**, 114 (1958)
56. Davenport, H. W., in *Metabolic Aspects of Transport across Cell Membranes*, 295 (Murphy, Q. R., Ed., University of Wisconsin Press, Madison, Wisconsin, 379 pp., 1957)
57. Davies, J., *Am. J. Physiol.*, **188**, 21 (1957)
58. Davies, R. E., in *Metabolic Aspects of Transport across Cell Membranes*, 277 (Murphy, Q. R., Ed., University of Wisconsin Press, Madison, Wis., 379 pp., 1957)
59. Davson, H., *Physiology of the Ocular and Cerebrospinal Fluids* (J. and A. Churchill, Ltd., London, Engl., 388 pp. 1956)
60. Davson, H., and Luck, C. P., *J. Physiol. (London)*, **132**, 454 (1956)
61. Davson, H., and Luck, C. P., *Am. J. Ophthalmol.*, **41**, 809 (1956)
62. Davson, H., and Luck, C. P., *J. Physiol. (London)*, **137**, 279 (1957)
63. Deyrup, I. J., *J. Gen. Physiol.*, **39**, 893 (1956)
64. Deyrup, I. J., *J. Gen. Physiol.*, **41**, 49 (1957)
65. Deyrup, I. J., *Am. J. Physiol.*, **188**, 125 (1957)
66. Dosekun, F. O., and Mendel, D., *J. Physiol. (London)*, **140**, 190 (1958)
67. Dray, S., and Sollner, K., *Biochim. et Biophys. Acta*, **21**, 126 (1956)
68. Dray, S., and Sollner, K., *Biochim. et Biophys. Acta*, **22**, 213, 220 (1956)
69. Dumont, P., *Arch. intern. pharmacodynamie*, **109**, 334 (1957)
70. Durbin, R. P., Frank, H., and Solomon, A. K., *J. Gen. Physiol.*, **39**, 535 (1956)
71. Eckel, R. E., Norris, J. E. C., and Pope, C. E., *Am. J. Physiol.*, **193**, 644 (1958)

72. Edwards, C., and Harris, E. J., *J. Physiol. (London)*, **135**, 567 (1957)
73. Edwards, C., Harris, E. J., and Nishie, K., *J. Physiol. (London)*, **135**, 560 (1957)
74. Eichelberger, L., Akeson, W. H., and Roma, M., *Am. J. Physiol.*, **185**, 287 (1956)
75. Eichelberger, L., Roma, M., and Moulder, P. V., *J. Appl. Physiol.*, **12**, 42 (1958)
76. Engbaek, L., and Hoshiko, T., *Acta Physiol. Scand.*, **39**, 348 (1957)
77. Evans, J. V., *J. Physiol. (London)*, **136**, 41 (1957)
78. Evans, J. V., and Phillipson, A. T., *J. Physiol. (London)*, **139**, 87 (1957)
79. Fisher, R. B., and Lindsay, D. B., *J. Physiol. (London)*, **131**, 526 (1956)
80. Fleckenstein, A., Gerlach, E., and Janke, J., *Schweiz. med. Wochschr.*, **86**, 1041 (1956)
81. Fleming, W. R., *J. Cellular Comp. Physiol.*, **49**, 129 (1957)
82. Forbes, G. B., *Proc. Soc. Exptl. Biol. Med.*, **98**, 153 (1958)
83. Forbes, G. B., and Lewis, A. M., *J. Clin. Invest.*, **35**, 596 (1957)
84. Forbes, G. B., Mizner, G. L., and Lewis, A., *Am. J. Physiol.*, **190**, 152 (1957)
85. Foulkes, E. C., *J. Gen. Physiol.*, **39**, 687 (1956)
86. Foulkes, E. C., and Miller, B. F., *Arch. Biochem. Biophys.*, **73**, 327 (1958)
87. Fuchs, F., and Fuchs, A.-R., *Acta Physiol. Scand.*, **38**, 379, 391 (1957)
88. Gamble, J. L., Jr., *J. Biol. Chem.*, **228**, 955 (1957)
89. Garby, L., *Acta Physiol. Scand.*, **40**, Suppl. 137, 1-84 (1957)
90. Garby, L., and Ulfendahl, H., *Acta Physiol. Scand.*, **35**, 167 (1955)
91. Gárdos, G., and Straub, F. B., *Acta physiol. hung.*, **12**, 1 (1957)
92. Gaudino, M., *Am. J. Physiol.*, **187**, 75 (1956)
93. Gerlach, E., Fleckenstein, A., and Gross, E., *Arch. ges. Physiol.*, **266**, 528 (1958)
94. Giebisch, G., Kraupp, O., Pillat, B., and Stormann, H., *Experientia*, **13**, 443 (1957)
95. Gilbert, D. L., and Fenn, W. O., *J. Gen. Physiol.*, **40**, 393 (1957)
96. Glynn, I. M., *J. Physiol. (London)*, **134**, 278 (1956)
97. Glynn, I. M., *J. Physiol. (London)*, **136**, 148 (1957)
98. Glynn, I. M., *Prog. in Biophys. and Biophys. Chem.*, **8**, 243 (1957)
99. Gollan, F., Rudolph, G. G., and Olsen, N. S., *Am. J. Physiol.*, **189**, 277 (1957)
100. Gonlubol, F., Siegel, A., and Bing, R. J., *Circulation Research*, **4**, 298 (1956)
101. Goodman, J., and Rothstein, A., *J. Gen. Physiol.*, **40**, 915 (1957)
102. Gosselin, R. E., *J. Gen. Physiol.*, **39**, 625 (1956)
103. Gourley, D. R. H., *Am. J. Physiol.*, **190**, 536 (1957)
104. Green, H., Mann, M. J., and Kroman, H. S., *Am. J. Ophthalmol.*, **44**, Part II, 388 (1957)
105. Greig, M. E., and Gibbons, A. J., *Arch. Biochem. Biophys.*, **61**, 343 (1956)
106. Grim, E., and Smith, G. A., *Am. J. Physiol.*, **191**, 555 (1957)
107. Grim, E., and Sollner, K., *J. Gen. Physiol.*, **40**, 887 (1957)
108. Griswold, R. L., and Pace, N., *Exptl. Cell Research*, **11**, 362 (1956)
109. Grob, D., Johns, R. J., and Liljestrand, Å., *Am. J. Med.*, **23**, 356 (1957)
110. Grob, D., Liljestrand, Å., and Johns, R. J., *Am. J. Med.*, **23**, 340 (1957)
111. Gunn, S. A., and Gould, T. C., *Proc. Soc. Exptl. Biol. Med.*, **96**, 820 (1957)
112. Hall, P. F., and Myant, N. B., *J. Physiol. (London)*, **133**, 181 (1956)
113. Harris, E. J., *J. Gen. Physiol.*, **41**, 169 (1957)
114. Harris, E. J., *Biochim. et Biophys. Acta*, **23**, 80 (1957)
115. Harris, E. J., and Prankerd, T. A. J., *J. Gen. Physiol.*, **41**, 197 (1957)
116. Harris, E. J., and Steinbach, H. B., *J. Physiol. (London)*, **133**, 385 (1956)

117. Harris, J. E., Carlson, A. E., Gruber, L., and Hoskinson, G., *Am. J. Ophthalmol.*, **44**, Part II, 409 (1957)
118. Heinz, E., *J. Biol. Chem.*, **225**, 305 (1957)
119. Heinz, E., and Mariani, H. A., *J. Biol. Chem.*, **228**, 97 (1957)
120. Helmreich, E., and Cori, C. F., *J. Biol. Chem.*, **224**, 663 (1957)
121. Hempling, H. G., *J. Gen. Physiol.*, **41**, 565 (1958)
122. Herlin, L., *Acta Physiol. Scand.*, **37**, Suppl. 127 (1956)
123. Herold, L., *Arch. Gynäkol.*, **187**, 388 (1956)
124. Heyndrickx, G. V., and Peeters, G., *Quart. J. Exptl. Physiol.*, **43**, 174 (1958)
125. Hirvonen, L., and Lybeck, H., *Acta Physiol. Scand.*, **36**, 17 (1956)
126. Hodgkin, A. L., *Proc. Roy. Soc. (London)*, **B148**, 1 (1958)
127. Hodgkin, A. L., and Keynes, R. D., *J. Physiol. (London)*, **138**, 253 (1957)
128. Holland, W. C., and Auditore, G. V., *J. Appl. Physiol.*, **9**, 147 (1956)
129. Hollander, W., Jr., Winters, R. W., Williams, T. F., Bradley, J., Oliver, J., and Welt, L. G., *Am. J. Physiol.*, **189**, 557 (1957)
130. Holliday, M. A., and Segar, W. E., *Am. J. Physiol.*, **191**, 610 (1957)
131. Holliday, M. A., Segar, W. E., Lukenbill, A., Valencia, R. M., and Durrell, A. M., *Proc. Soc. Exptl. Biol. Med.*, **95**, 786 (1957)
132. Holmberg, N. G., Kaplan, B., Karvonen, M. J., Lind, J., and Malm, M., *Acta Physiol. Scand.*, **36**, 291 (1956)
133. Hong, S. K., *Am. J. Physiol.*, **188**, 439 (1957)
134. Hsu, J. M., Davis, R. L., and Chow, B. F., *J. Biol. Chem.*, **230**, 889 (1958)
135. Huf, E. G., Doss, N. S., and Wills, J. P., *J. Gen. Physiol.*, **41**, 397 (1957)
136. Iacobellis, M., Griffin, G. E., and Muntwyler, E., *Proc. Soc. Exptl. Biol. Med.*, **96**, 64 (1957)
137. Iacobellis, M., Muntwyler, E., and Dodgen, C. L., *Am. J. Physiol.*, **185**, 275 (1956)
138. Itoh, S., and Schwartz, I. L., *J. Gen. Physiol.*, **40**, 171 (1956)
139. Itoh, S., and Schwartz, I. L., *Am. J. Physiol.*, **188**, 490 (1957)
140. Jackson, K. L., and Pace, N., *J. Gen. Physiol.*, **40**, 47 (1956)
141. Jardetzky, O., *Science*, **125**, 931 (1957)
142. Jennings, D., Hooper, D., and Rothstein, A., *J. Gen. Physiol.*, **41**, 1019 (1958)
143. Johnson, J. A., *Am. J. Physiol.*, **187**, 328 (1956)
144. Johnson, T. H., *Am. J. Obstet. Gynecol.*, **75**, 240 (1958)
145. Jojima, T., and Kuriyama, H. A., *Japan. J. Physiol.*, **7**, 347 (1957)
146. Kahn, J. B., Jr., *J. Pharmacol. Exptl. Therap.*, **120**, 239 (1957)
147. Kahn, J. B., Jr., *J. Pharmacol. Exptl. Therap.*, **121**, 234 (1957)
148. Kahn, J. B., Jr., and Cohen, S. B., *J. Pharmacol. Exptl. Therap.*, **120**, 9 (1957)
149. Kalman, S. M., *J. Pharmacol. Exptl. Therap.*, **121**, 252 (1957)
150. Kalman, S. M., and Lowenstein, J. M., *J. Pharmacol. Exptl. Therap.*, **122**, 163 (1958)
151. Kaplan, S. A., and Stein, S. N., *Am. J. Physiol.*, **190**, 163 (1957)
152. Kaplan, S. A., and Stein, S. N., *Am. J. Physiol.*, **190**, 166 (1957)
153. Kärki, N., Burn, G. P., and Burn, J. H., *Lancet*, **I**, 565 (1957)
154. Kato, H. P., Zwolinski, B. J., and Eyring, H., *J. Phys. Chem.*, **60**, 404 (1956)
155. Kedem, O., and Katchalsky, A., *Biochim. et Biophys. Acta*, **27**, 229 (1957)
156. Keynes, R. D., and Lewis, P. R., *J. Physiol. (London)*, **134**, 399 (1956)

157. Kipnis, D. M., and Cori, C. F., *J. Biol. Chem.*, **224**, 681 (1957)
158. Kirschner, L. B., *Arch. Biochem. Biophys.*, **68**, 499 (1957)
159. Kleinzeller, A., and Cort, J. H., *Biochem. J.*, **67**, 15 (1957)
160. Kleinzeller, A., and Cort, J. H., *Nature*, **180**, 1124 (1957)
161. Koch, H. J., and Evans, J., *Mededel. Koninkl. Vlaam. Acad. Wetenschap. Belg.*, **18**, No. 6 (1956)
162. Koch, H. J., and Evans, J., *Mededel. Koninkl. Vlaam. Acad. Wetenschap. Belg.*, **18**, No. 7 (1956)
163. Koch, H. J., and Evans, J., *Mededel. Koninkl. Vlaam. Acad. Wetenschap. Belg.*, **18**, No. 8 (1956)
- 163a. Koefoed-Johnsen, V., and Ussing, H. H., *Acta Physiol. Scand.*, **42**, 298 (1958)
164. Krahle, M. E., *Perspectives in Biol. & Med.*, **1**, 69 (1957)
165. Kunz, H. A., and Sulser, F., *Experientia*, **13**, 365 (1957)
166. Lajtha, A., *J. Neurochem.*, **1**, 216 (1957)
167. Langham, M. E., *Physiol. Revs.*, **38**, 215 (1958)
168. Langham, M. E., and Lee, P. M., *Brit. J. Ophthalmol.*, **41**, 65 (1957)
169. Leaf, A., *Biochem. J.*, **62**, 241 (1956)
170. Leaf, A., Anderson, J., and Page, L. B., *J. Gen. Physiol.*, **41**, 657 (1958)
171. Leaf, A., and Renshaw, A., *Biochem. J.*, **65**, 82 (1957)
172. Leaf, A., and Renshaw, A., *Biochem. J.*, **65**, 90 (1957)
173. Leake, C. D., and Pomerat, C. M., *Science*, **127**, 162 (1958)
174. Lifson, N., and Parsons, D. S., *Proc. Soc. Exptl. Biol. Med.*, **95**, 532 (1957)
175. Ling, G., in *Metabolic Aspects of Transport across Cell Membranes*, 181 (Murphy, Q. R., Ed., University of Wisconsin, Press, Madison, Wis., 379 pp., 1957)
176. Logothetopoulos, J. H., and Myant, N. B., *J. Physiol. (London)*, **134**, 189 (1956)
177. Logothetopoulos, J. H., and Scott, R. F., *J. Physiol. (London)*, **132**, 365 (1956)
178. Lubin, M., and Schneider, P. B., *J. Physiol. (London)*, **138**, 140 (1957)
179. Lundberg, A., *Physiol. Revs.*, **38**, 21 (1958)
180. Lybeck, H., *Acta Physiol. Scand.*, **37**, 215 (1956)
181. McHardy, G. J. R., and Parsons, D. S., *Quart. J. Exptl. Physiol.*, **42**, 33 (1957)
182. McLennan, H., *Biochem. et Biophys. Acta*, **21**, 472 (1956)
183. McLennan, H., *Biochim. et Biophys. Acta*, **22**, 30 (1956)
184. McLennan, H., *Biochim. et Biophys. Acta*, **24**, 1 (1957)
185. McLennan, H., *Biochim. et Biophys. Acta*, **24**, 333 (1957)
186. McLennan, H., *Biochim. et Biophys. Acta*, **27**, 624 (1958)
187. Maizels, M., *J. Physiol. (London)*, **132**, 414 (1956)
188. Maizels, M., Remington, M., and Truscoe, R., *J. Physiol. (London)*, **140**, 48 (1958)
189. Maizels, M., Remington, M., and Truscoe, R., *J. Physiol. (London)*, **140**, 61 (1958)
190. Maizels, M., Remington, M., and Truscoe, R., *J. Physiol. (London)*, **140**, 80 (1958)
191. Manery, J. F., Gourley, D. R. H., and Fisher, K. C., *Can. J. Biochem. and Physiol.*, **34**, 893 (1956)
192. Margoshes, M., and Vallee, B. L., *Methods of Biochem. Anal.*, **3**, 353 (1956)
193. Mark, L. C., Burns, J. J., Campomanes, C. I., Ngai, S. H., Trousof, N., Papper, E. M., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, **119**, 35 (1957)
194. Martin, L., *Trans. Am. Clin. Climatol. Assoc.*, **67**, 25 (1956)
195. Maurice, D. M., *J. Physiol. (London)*, **137**, 110 (1957)

196. Mauro, A., *Science*, **126**, 252 (1957)
197. Mitchell, P., *Nature*, **180**, 134 (1957)
198. Mulder, A. G., Omachi, A., and Rebar, B. T., *Am. J. Physiol.*, **186**, 309 (1956)
199. Munro, D. S., Satoskar, R. S., and Wilson, G. M., *J. Physiol. (London)*, **139**, 474 (1957)
200. Murphy, Q. R., Ed., *Metabolic Aspects of Transport across Cell Membranes* (University of Wisconsin Press, Madison, Wis., 379 pp., 1957)
201. Nevis, A. H., *J. Gen. Physiol.*, **41**, 927 (1958)
202. Nichols, N., *Proc. Soc. Exptl. Biol. Med.*, **97**, 363 (1958)
203. Nichols, G., Jr., and Nichols, N., *Am. J. Physiol.*, **186**, 383 (1956)
204. Nichols, G., Jr., and Nichols, N., *Metabolism, Clin. and Exptl.*, **5**, 438 (1956)
205. Noall, M. W., Riggs, T. R., Walker, L. M., and Christensen, H. N., *Science*, **126**, 1002 (1957)
206. Öbrink, K. J., *Acta Physiol. Scand.*, **36**, 229 (1956)
207. Öbrink, K. J., *Ann. Rev. Physiol.*, **20**, 377 (1958)
208. Oliver, J., MacDowell, M., Welt, L. G., Holliday, M. A., Hollander, W., Jr., Winters, R. W., Williams, T. F., and Segar, W. E., *J. Exptl. Med.*, **106**, 563 (1957)
209. Opie, E. L., *J. Exptl. Med.*, **103**, 351 (1956)
210. Paganelli, C. V., and Solomon, A. K., *J. Gen. Physiol.*, **41**, 259 (1957)
211. Pappius, H. M., and Elliott, K. A. C., *Can. J. Biochem. and Physiol.*, **34**, 1007 (1956)
212. Pappius, H. M., and Elliott, K. A. C., *Can. J. Biochem. and Physiol.*, **34**, 1053 (1956)
213. Pappius, H. M., Rosenfeld, M., Johnson, D. McL., and Elliott, K. A. C., *Can. J. Biochem. and Physiol.*, **36**, 217 (1958)
214. Park, C. R., Johnson, L. H., Wright, J. H., Jr., and Batsel, H., *Am. J. Physiol.*, **191**, 13 (1957)
215. Paul, W. M., Enns, T., Reynolds, S. R. M., and Chinard, F. P., *J. Clin. Invest.*, **35**, 634 (1956)
216. Petit, G., *Biol. méd. (Paris)*, **46**, 40 (1957); **46**, 97 (1957)
217. Pickles, V. R., *J. Physiol. (London)*, **138**, 495 (1957)
218. Pillat, B., Kraupp, O., Giebisch, G., and Stormann, H., *Arch. ges. Physiol.*, **266**, 459 (1958)
219. Post, R. L., and Jolly, P. C., *Biochim. et Biophys. Acta*, **25**, 118 (1957)
220. Price, C. A., Fonnesu, A., and Davies, R. E., *Biochem. J.*, **64**, 754 (1956)
221. Quadbeck, G., and Helmchen, H., *Deut. med. Wochschr.*, **82**, 1377 (1957)
222. Randle, P. J., and Smith, G. H., *Biochim. et Biophys. Acta*, **25**, 442 (1957)
223. Rahman, M. H., Frazier, L. E., Hughes, R. H., and Cannon, P. R., *Arch. Pathol.*, **63**, 154 (1957)
224. Rayner, B., and Weatherall, M., *Brit. J. Pharmacol.*, **12**, 371 (1957)
225. Regan, T. J., Talmers, F. N., and Hellem, H. K., *J. Clin. Invest.*, **35**, 1220 (1956)
226. Rehm, W. S., and Dennis, W. H., in *Metabolic Aspects of Transport across Cell Membranes*, 303 (Murphy, Q. R., Ed., University of Wisconsin Press, Madison, Wis., 379 pp., 1957)
227. Relman, A. S., *Yale J. Biol. and Med.*, **29**, 248 (1956)
228. Relman, A. S., Lambie, A. T., Burrows, B. H., and Roy, A. M., *J. Clin. Invest.*, **36**, 1249 (1957)
229. Renkin, E. M., *J. Gen. Physiol.*, **38**, 225 (1954)

230. Renkin, E. M., *Natl. Acad. of Sci.—Natl. Research Council, Publ. No. 451*, 32 (1956)
231. Resnick, O., and Hechter, O., *J. Biol. Chem.*, **224**, 941 (1957)
232. Riecker, G., Zack, W., and Renschler, H. E., *Arch. ges. Physiol.*, **264**, 245 (1957)
233. Rixon, R. H., and Stevenson, J. A., *Can. J. Biochem. and Physiol.*, **34**, 1069 (1956)
234. Rixon, R. H., and Stevenson, J. A., *Quart. J. Exptl. Physiol.*, **42**, 346 (1957)
235. Robertson, J. S., *Physiol. Revs.*, **37**, 133 (1957)
236. Robertson, J. S., Tosteson, D. C., and Gamble, J. L., Jr., *J. Lab. Clin. Med.*, **49**, 497 (1957)
237. Robinson, J. R., *J. Physiol. (London)*, **134**, 216 (1956)
238. Robinson, J. R., *J. Physiol. (London)*, **136**, 585 (1957)
239. Rogers, T. A., *Am. J. Physiol.*, **191**, 363 (1957)
240. Rosenberg, T., and Wilbrandt, W., *J. Gen. Physiol.*, **41**, 289 (1957)
241. Ross, E. J., *Medicine*, **35**, 355 (1956)
242. Rothstein, A., and Hayes, A. D., *Arch. Biochem. Biophys.*, **63**, 87 (1956)
243. Rothstein, A., Hayes, A. D., Jennings, D., and Hooper, D., *J. Gen. Physiol.*, **41**, 585 (1958)
244. Rudolph, G. G., and Olsen, N. S., *Am. J. Physiol.*, **185**, 157 (1956)
245. Sacks, J., and Bakshy, S., *Am. J. Physiol.*, **189**, 339 (1957)
246. Sacks, J., and Smith, J. F., *Am. J. Physiol.*, **192**, 287, (1958)
247. Sawyer, W. H., and Schisgall, R. M., *Am. J. Physiol.*, **187**, 312 (1956)
248. Schmidt-Nielsen, K., Jørgensen, C. B., and Osaki, H., *Am. J. Physiol.*, **193**, 101 (1958)
249. Schoffeniels, E., *Arch. intern. physiol.*, **64**, 571 (1956)
250. Schoffeniels, E., *Biochim. et Biophys. Acta*, **26**, 585 (1957)
251. Schreiber, S. S., *Am. J. Physiol.*, **185**, 337 (1956)
252. Segal, S., Wyngaarden, J. B., and Foley, J., *J. Clin. Invest.*, **36**, 1383 (1957)
253. Shanes, A. M., *Pharmacol. Revs.*, **10**, 59 (1958)
254. Shaw, F. H., Simon, S. E., and Johnstone, B. M., *J. Gen. Physiol.*, **40**, 1 (1956)
255. Shaw, F. H., Simon, S. E., Johnstone, B. M., and Holman, M. E., *J. Gen. Physiol.*, **40**, 263 (1956)
256. Shaw, T. I., *J. Physiol. (London)*, **129**, 464 (1955)
257. Sherwood Jones, E., *Experientia*, **14**, 72 (1958)
258. Sidel, V. W., and Solomon, A. K., *J. Gen. Physiol.*, **41**, 243 (1957)
259. Simon, S. E., Shaw, F. H., Bennett, S., and Muller, M., *J. Gen. Physiol.*, **40**, 753 (1957)
260. Slobody, L. B., Yang, D. C., Lending, M., Borrelli, F. J., and Tyree, M., *Am. J. Physiol.*, **190**, 365 (1957)
261. Snell, F. M., and Leeman, C. P., *Biochim. et Biophys. Acta*, **25**, 311 (1957)
262. Solomon, A. K., Gill, T. J., 3rd, and Gold, G. L., *J. Gen. Physiol.*, **40**, 327 (1956)
263. Solomon, A. K., Lionetti, F., and Curran, P. F., *Nature*, **178**, 582 (1956)
264. Solomon, S., *J. Cellular Comp. Physiol.*, **49**, 351 (1957)
265. Stolkowski, J., and Reinberg, A., *Ann. endocrinol. (Paris)*, **17**, 137 (1956)
266. Sutcliffe, J. F., and Hackett, D. P., *Nature*, **180**, 96 (1957)
267. Szent-Györgyi, A., *Bioenergetics*, 80 (Academic Press, Inc., New York, N. Y.,* 143 pp., 1957)
268. Talso, P. J., Strub, I. H., and Kirsner, J. B., *J. Lab. Clin. Med.*, **47**, 210 (1956)

269. Tashiro, Y., *Acta Schol. Med., Univ. Kioto*, **34**, 123, 130 (1957)
270. Tashiro, Y., *Acta Schol. Med., Univ. Kioto*, **34**, 140 (1957)
271. Taubenhaus, M., and Morton, J. V., *Proc. Soc. Exptl. Biol. Med.*, **98**, 163 (1958)
272. Taylor, I. M., *Natl. Acad. Sci.—Natl. Research Council, Publ. No. 451*, **26**, (1956)
273. Thomas, J., and McIlwain, H., *J. Neurochem.*, **1**, 1 (1956)
274. Thomason, D., and Schofield, R., *Nature*, **181**, 1207 (1958)
275. Tobian, L., *Circulation Research*, **4**, 671 (1956)
276. Tobian, L., and Fox, A., *J. Clin. Invest.*, **35**, 297 (1956)
277. Tobian, L., and Redleaf, P. D., *Am. J. Physiol.*, **189**, 451 (1957)
278. Tobian, L., and Redleaf, P. D., *Am. J. Physiol.*, **192**, 325 (1958)
279. Tosteson, D. C., *J. Cellular Comp. Physiol.*, **50**, 199 (1957)
280. Tosteson, D. C., and Johnson, J., *J. Cellular Comp. Physiol.*, **50**, 169 (1957)
281. Tosteson, D. C., and Johnson, J., *J. Cellular Comp. Physiol.*, **50**, 185 (1957)
282. Ussing, H. H., *Ion Transport across Membranes*, 3 (Clarke, H. T., Ed., Academic Press, Inc., New York, N. Y., 298 pp., 1954)
283. Ussing, H. H., in *Metabolic Aspects of Transport across Cell Membranes*, 39 (Murphy, Q. R., Ed., University of Wisconsin Press, Madison, Wis., 379 pp., 1957)
284. Van der Kloot, W. G., *J. Gen. Physiol.*, **41**, 879 (1958)
285. Vick, R. L., and Kahn, J. B., Jr., *J. Pharmacol. Exptl. Therapy.*, **121**, 389 (1957)
286. Whittam, R., *J. Physiol. (London)*, **140**, 479 (1958)
287. Wilbrandt, W., *Deut. med. Wochschr.*, **82**, 1153 (1957)
288. Woodbury, D. M., *Am. J. Physiol.*, **185**, 281 (1956)
289. Woodbury, D. M., and Koch, A., *Proc. Soc. Exptl. Biol. Med.*, **94**, 720 (1957)
290. Woodbury, D. M., Rollins, L. T., Gardner, M. D., Hirschi, W. L., Hogan, J. R., Rallison, M. L., Tanner, H. S., and Brodie, D. A., *Am. J. Physiol.*, **192**, 79 (1958)
291. Zerahn, K., *Acta Physiol. Scand.*, **36**, 300 (1956)
292. Zierler, K. L., *Am. J. Physiol.*, **185**, 1 (1956)
293. Zierler, K. L., *Am. J. Physiol.*, **190**, 201 (1957)
294. Zierler, K. L., *Science*, **126**, 1067 (1957)
295. Zierler, K. L., *Am. J. Physiol.*, **192**, 283 (1958)
296. Zierler, K. L., and Andres, R., *J. Clin. Invest.*, **36**, 730 (1956)

CHEMICAL INFLUENCES ON CELL DIVISION AND DEVELOPMENT¹

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It would be virtually impossible in a short review of this nature to attempt a complete analysis of all contributions to the area of chemical effects on mitosis and cell development. An arbitrary emphasis, therefore, has been placed on the effects of drugs on mitosis. An effort has been made to select the most significant contributions to this field. Because of the extensive rapidly accumulating literature even in this more limited area, the reviewer has chosen to emphasize primarily those investigations which, at least in part, are directed to elaboration of mechanisms. This arbitrary selection has made it necessary to leave out references to many worthy research efforts. The review also has been limited in another direction. There is a growing interest in compounds that stimulate mitosis particularly in plants. The gibberellins have been more completely studied than any other agents, and are considered below. Discussion of other plant hormones and mitotic stimulants have been omitted, not because they are not important but because they are less completely evaluated by current reports than the gibberellins.

COLCHICINES AND RELATED DERIVATIVES

Of all the chemical substances used in studying the effects of drugs on mitosis, perhaps no agent has been more completely investigated than colchicine, a natural chemical derived from *Colchicum autumnale*. Several reviews (1, 2, 3) have appeared which relate the early history, the chemistry, and the therapeutic applications of colchicine. Dustin (1) at first, in 1934, believed that colchicine's action on cells was a stimulating one causing an increased number of mitoses, a logical conclusion at the time. Ludford's (4) position on the basis of later experimental findings was that colchicine exerted its influence primarily upon the cytoplasm rather than directly upon the nucleus and moreover was not primarily a drug stimulating mitosis but one which allowed the accumulation of mitotic figures. This latter position has been reiterated and confirmed many times. Because many other drugs have been shown to be mitotic inhibitors only in high concentrations or only under certain special circumstances, colchicine has remained a general standard of comparison for all antimitotic drugs because it inhibits at extremely low concentrations and is effective, either *in vitro* or *in vivo* or both, in most plant and animal cells. Colchicine appears to owe its major activity to an effect upon the viscosity of the cytoplasm. This was first indicated by

¹ The survey of the literature pertaining to this review was concluded April 30, 1958.

Beams & Evans in 1940 (5). Their observations based on the rate of stratification in sea urchin eggs were confirmed and extended by Wilbur (6).

Although it has been known since 1934 (7) that colchicine produces a disorganization of the mitotic spindle (c-mitosis), it has been the more recent, elegant studies carried out in Mazia's laboratory (8, 9, 10) that have precisely indicated that this agent has its primary action on the organization rather than on the formation of the spindle. Mazia (8) suggests that the spindle is formed by polymerization of protein through formation of —S—S— bonds, whereas secondary bonding (H bonding) is responsible for the organization of the mitotic apparatus. There is some evidence that the chromosomes (centromeres) and the centrosomes contribute to this secondary bonding. Only the latter process seems to be modified by colchicine, since the mitotic apparatus can be isolated in an amorphous gel from colchicine-treated sea urchin eggs. There is no evidence that normal chromosomal duplication is modified in any way. Although the effect of colchicine on mitosis has been more thoroughly studied than that of any other chemical, there is a discouraging lack of data on its biochemical mechanism of action. And there is even less known about many other agents which produce c-mitosis (11). It is, however, of interest that some of these metaphase inhibitors are capable of combining with —SH groups (4, 12, 13, 14). On the other hand, amino acid analogues of phenylalanine are metaphase inhibitors but are not —SH inhibitors (16). Although the evidence that certain metaphase inhibitors combine with protein —SH is reasonably convincing, it is by no means established that this is the only mechanism of modifying the developing mitotic apparatus. Any one or several other chemical mechanisms could be involved. Lettré (15, 17) for example argues, but not convincingly, that colchicine acts by preventing the utilization of ATP in chromosome movement. A-methopterin and aminopterin (18) which modify purine synthesis produce cellular effects which closely resemble those of colchicine. As chromosome duplication progresses normally, it has been suggested by Swann (19) that it is nuclear RNA synthesis that is modified making it unavailable for release in adequate concentrations to participate effectively in the formation of the mitotic spindle. The magnitude of the problem of determining mechanisms of the action of such drugs is readily appreciated when the complexities of mitosis are considered (8, 20, 21, 22).

The hypothesis that the effect of colchicine on mitosis results from a reduction of cytoplasmic viscosity is further strengthened by the observations of Nishimura & Baum (23, 24) who showed that when resistance of Ehrlich ascites tumor to colchicine occurred, viscosity of the cytoplasm was no longer modified.

To attribute all of the actions of colchicine to an effect on changes in viscosity and secondary bonding is probably not justified. Padawer & Gordon (25), studying mast cells in peritoneal fluid of rats treated subcutaneously with colchicine, observed fragmentation of the cells without any noticeable change in the nuclei one hour after administration of sublethal doses of

the drug. It has, moreover, been shown that colchicine in small doses (0.0001 per cent) can act as a plant cell stimulant (26). Also colchicine in small doses has a minor effect on the cell in preprophase. This, however, is overshadowed by the more prominent action during metaphase.

It appears from the studies of Swann (19) that cell growth and mitosis may not be intimately related, beyond a required minimum of growth before cell division can take place. That cells exposed to mitotic inhibitors may continue to grow has also been reported. Gati *et al.* (27) have recently demonstrated that colchicine causes Ehrlich ascites carcinoma cells to grow larger, growth being accompanied by an increase in the size of their nuclei and nucleoli. Less extensive, but similar, effects are produced by podophyllin. This cell enlargement in the presence of mitotic inhibitors suggests that either directly or indirectly such agents modify the amount of some cellular component responsible for normal growth regulation. Gropp (28) has presented evidence from his studies of N-methyl-colchicinamide and 4'-azabenzopyrene, administered separately and in combination to mice bearing ascites tumors, that the division of the nucleus and plasma are two unrelated phenomena that are normally independently coordinated in time and that under the influence of such chemical agents each process can be differentially inhibited. Further reference will be made to this phenomenon in another part of this review.

Lettré (17) has reviewed the effectiveness of many compounds chemically related to colchicine. Many colchicine derivatives are mitotic inhibitors differing from the parent compound quantitatively rather than qualitatively. Resegatti & von Euler (29) have shown in rat ascites tumor that the d-tartrate of the methyl ester of trimethyl colchicinic acid in small doses inhibits mitosis in seven hours and in medium doses arrests it. As with colchicine, the action is enhanced by phlorhizin. Truhaut & Deysson (30), using roots of *Allium cepa*, demonstrated that colchicine and N-deacetylthiocolchicine were nearly twice as potent mitoclastic agents as colchicine. Aminocolchimidazole (31) in a concentration of 2.5×10^{-7} M causes a disappearance of mitosis in the roots of *Pisum* in two days. Fragmentation and agglutination of the chromosomes were observed, distinguishing its action in this respect from that of colchicine. Its action more closely resembled that of guanidine than colchicine. The actions of colchicine and desacetylcolchicine on embryonic myocardial chick tissue in hanging drop cultures (32) were compared and the latter found to be a distinctly more potent antimitotic inhibitor. The increase in metaphase figures was clearly demonstrated by the end of the first hour.

Linskens & Wulf (33) prepared lumicolchicines and, after keeping the irradiated material in the dark for one year, separated the products in a 3.5×15 cm. alumina column in an ether solvent. The fractions were tested on germinating cress and *Vicia* root tips and found to lack mitosis-inhibiting activity. Desacetylmethylcolchicine (Colcemid) administered to rats (34) masked the 24-hr. mitotic periodicities in buccal epithelium, duodenal crypts,

thyroid glands, and adrenal cortices. The predominant actions seemed to be a preprophase and a metaphase inhibition of mitosis.

The action of colchicine is obviously a rather complicated one although the work of Mazia and others (8, 9, 10) strongly indicates that we are approaching, albeit slowly, a biochemical explanation of its action. Lest we become too optimistic, we should recall the observations of Benitez, Murray & Chargaff (35) that three diverse agents, mesoinositol, adenosinetriphosphate, and tropolone, will reverse the metaphase arrest of rat fibroblasts produced by colchicine.

As Gross emphasizes repeatedly (20), mitosis is dependent upon gelation of the cytoplasm, and any factors modifying the gelation of the cytoplasm would, therefore, directly or indirectly modify the initiation or development of the mitotic process. Although the nature of the gelation process is still obscure, many careful and elaborate studies have been performed to help unravel the nature of this important cellular phenomenon. Heilbrunn (36) in a very extensive monograph on cellular sol-gel transformations develops the thesis, rather convincingly, that shifts in the localization of ionic calcium within the cell are determining factors. He has found that stimulation of the cell is accompanied by the release of Ca^{++} from a bound state and that this free, unbound calcium produces a gelation of the endoplasm. Heilbrunn, however, does not supply evidence that there are significant changes in ion binding capacity which would be necessary for calcium release. Gross, on the other hand, believes that mechanisms for accomplishing such changes in cation binding power of proteins are readily available, as indicated by the fact that stimulants can reduce the availability of anionic sites on cortical proteins. He also reminds us of the fact that spermatozoa bring strongly basic proteins to the cell and he believes that these polycations could compete with calcium ion for ionized carboxylate sites on protein which modify the preference for one cationic site over another. Gross points out

the point of general agreement is that sol-gel transformation in the cytoplasm must reflect the lability of certain colloidal complexes among which association-dissociation equilibria exist and that shifts in the position of equilibrium determine the colloidal properties of the cytoplasm. Such shifts depend, in turn, upon the availability of the diffusible reactants possessing the power to alter charge or steric configuration on the surfaces of the sensitive macromolecule.

Hultin (37, 38) has presented some very convincing data in favor of the calcium ion hypothesis. He prepared breis of decalcified sea urchin eggs to which calcium ion was added. This produced a series of biochemical events which closely resemble those accompanying fertilization or stimulation of the normal cell: to wit, an increased uptake of oxygen; production of acid; unmasking of $-\text{SH}$ groups; lysis of cytoplasmic inclusions; and, of particular interest to us in this discussion, an increase in viscosity. Gross (39, 40) in a series of studies on *Arabacia punctulata*, has confirmed these observations of Hultin.

The work of Schneider (41) adds further substantiation to the hypothesis that calcium ion plays a significant role in sol-gel formations, showing that calcium ion is a precipitant of nucleoproteins of rat liver. Gross carried these

observations somewhat further employing a more convincing technique. He perfused mammalian livers with citrate containing calcium ion-free media to wash all blood from the tissue. He then carried out experiments, comparable to those employed with sea urchin eggs, on the breis of the rat liver and demonstrated that Ca^{++} addition resulted in a loss of solubility of protein and RNA.

In further studies on pure microsomal preparations Gross was able to show that calcium ion produced aggregation which was associated with the considerable drop in pH. He concludes that the *in vivo* constituents most likely to react with calcium as a colloidal reactant are cytoplasmic granules which he believes may be identical with the microsomal particle. These observations closely approximate the changes taking place during mitosis which have been described by Mazia and referred to above, the first event being the generalized cytoplasmic gelation. Although Mazia, in his isolated spindles, found no nucleoprotein, it would seem on the basis of rather extensive cytochemical studies that the nucleoprotein may have been extracted in the isolation procedure. It is hypothesized by Gross that the spindle is formed from an aggregation of small particles, either microsomes or microsome-like bodies in the cytoplasm.

This rather brief review indicates the many complexities in our current state of knowledge of the cytoplasmic gelation which make it apparent that there are several stages at which an antimitotic substance might act in the prevention of formation of the mitotic apparatus independent of any effect upon the chromosome itself. Perhaps, until we understand better the sol-gel interrelationships within the cytoplasm and the detailed chemistry of spindle formation, it will not be possible to localize precisely the action of many antimitotic drugs, either of the colchicine type producing c-mitosis, or of other agents which may interfere in earlier stages of spindle formation.

ALKYLATING AGENTS

As described by Price (42), alkylation can be simply defined as $\text{H}-\text{M} \rightarrow \text{RX}$. Those alkylations of interest to us here apparently all involve attachment of the alkyl group to X through an oxygen, nitrogen, or sulfur atom. Ross (43) has concluded that because of the wide variety of structures of alkylating agents it is unlikely that they are effective by any process of competitive inhibition or physical absorption. He classifies these agents as follows: sulfur mustards, nitrogen mustards (HN_2), epoxides, ethyleneimines (TEPA), methane-sulfonates (Myleran), β -lactones and possibly certain diazo compounds (Azaserine). For a description of the chemical reactions of the alkylating agents, the reader is directed to a recent paper by Ross (43). Although Alexander (44) has reviewed the reactions of the sulfur mustard $[\text{S}(\text{CH}_2\cdot\text{CH}_2\cdot\text{Cl})_2]$ with proteins and nucleic acids, it is difficult to evaluate the significance of the results when related to *in vivo* systems because of the large excess of drug present. Stacey *et al.* (45) have more recently attempted to reinvestigate the effects of certain alkylating agents, using concentrations of drug which could not be expected to fill all accessible sites on cell proteins,

nucleic acids, and nucleoproteins. Stacey and his colleagues found that, except for the epoxides, these agents preferentially esterify carboxyl groups in bovine albumin. The —SH groups in undenatured protein showed either slight or no reactions. It was concluded that the only reaction with native protein which was shared by all alkylating agents was esterification of carboxyl groups. Moreover, esterification of carboxyl groups produced in most instances a large percentage decrease in viscosity. Alexander (46) has shown that the phosphate group of DNA could be esterified by alkylating agents. Also, Stacey *et al.* (45) have shown that the aromatic nitrogen mustard (CB 3025), when used in the presence of an excess of DNA phosphate groups, reacts entirely by esterification. Any unreacted mustard was lost by hydrolysis. Kay *et al.* (47, 48) have shown similar esterification of RNA phosphate using L-phenylalanine mustard. Stacey *et al.* (45) have obtained similar results with deoxynucleoprotein. It is important to remember that intramolecular cross-linking can occur with polyfunctional alkylating agents—a reaction that leads to a reduction in viscosity. On the contrary, intermolecular linkages lead to an increase in molecular weight and increased viscosity. As the cytoplasmic viscosity is apparently intimately related to mitosis, these effects of alkylating agents are quite significant, particularly since intramolecular cross-linking is observed with lower drug concentrations. It is tempting to postulate that the first reaction of these agents is with cytoplasmic protein and RNA, resulting in reduced viscosity and subsequent mitotic inhibition and that with greater concentrations of the drug, molecular bridges are formed with the resulting chromosomal aberrations.

Hutchens & Podolsky (49) have shown that HN_2 in sublethal concentrations reduces temporarily the fission rate of *Chilomonas paramecium*. Cells which are unable to undergo fission continue to synthesize nucleic acids and proteins, again demonstrating the independence of growth and cell division. The temporary nature of fission arrest is possibly a result of an initial combination between HN_2 and an essential factor for fission. This essential factor is then synthesized and fission is again able to take place. Loveless (50) has shown that division of *E. coli B* can be inhibited by concentrations of HN_2 and triethylene melamine (TEM) that do not prevent growth. Katchman (51) showed that during this growth period DNA and RNA synthesis continued at nearly normal rate. Powers & Pomeroy (52) reviewed a series of papers by Ord, Danielli *et al.* in which they studied the effect of HN_2 on *Amoeba proteus*. Both treated and untreated nuclei were transferred to treated and untreated cytoplasm of the same species. Nuclei treated with HN_2 were still able to divide when transplanted to normal cytoplasm. They concluded that untreated cytoplasm was able to carry the cell through at least one division. When untreated nuclei were transferred to treated cytoplasm, none of the cells divided prior to death. On the unit weight basis the cytoplasm appeared more sensitive than the nucleus to the action of HN_2 . Powers & Ehret (53), studying the effect of HN_2 on *Paramecium aurelia*, have further confirmed the cytoplasmic importance to fission. Powers & Pomeroy (52) raise the troublesome question, "If the action of alkylating

agents in cells is a consequence of reaction between them and nucleic acids, why should protein synthesis, commonly believed to be under RNA control, continue in the cytoplasm with high HN_2 concentration?" For a comparison of the action of alkylating agents and x-radiation, the reader is referred to the recent paper of Powers & Pomeroy (52). Perhaps the observations of Roberts (54) are more in keeping with the actual intracellular reactions of alkylating agents. He has studied the metabolic products and distribution in the rat of labeled ethyl methanesulfonate (CB 1528). The C^{14} -labeled drug was injected intraperitoneally. Of particular interest was the chromatographic analysis of the first 24-hr. urine sample. The bulk of the radioactivity was in two sulfur-containing compounds, N-acetyl-S-ethyl cysteine and S-ethyl cysteine. Roberts found no labeled carboxylic or phosphoric acid esters and therefore concluded that the principal reaction of the alkylating agent *in vivo* was with —SH groups. Whatever the mechanism may eventually prove to be, Alexander & Stacey (55) have shown that only about 30 out of 10^4 DNA molecules or 30 out of 10^6 protein molecules must be alkylated to produce cellular toxicity. Therefore the sites involved must be critical and specific ones. If Roberts is correct, this then would indicate that the —SH groups involved are few and specific, leaving many uninvolved —SH groups which by the nature of their position in the macromolecules are not available for alkylation; this, however, does not imply that they are not available for combination with other —SH inhibitors. Although the relative *in vivo* importance of blocking DNA phosphate groups, protein carboxyl groups, and protein —SH groups remains unanswered, the recent investigations reported above clearly bring us much closer to an understanding of the chemistry involved when cell constituents react with alkylating agents.

ANTIFOLIC ACIDS AND AMINO ACIDS

It has been repeatedly established that A-methopterin (4-amino-N-methylpteryolglutamic acid) is an antagonist of folic acids and citrovorum factor (56, 57). However, the mechanism by which this compound interferes with mitotic growth has not been elaborated. Barton & Laird (58) have compared the relative effects of A-methopterin on cells which are not rapidly mitosing with those of rapidly mitosing cells. Changes in RNA, protein, and DNA under these two conditions were recorded. They measured the incorporation of glycine- C^{14} and inorganic P^{32} into certain cell constituents of rat liver following partial hepatectomy, which is followed by rapid mitotic growth, and in cell growth following extended period of fasting where growth is unaccompanied by mitosis. Nucleic acid synthesis was inhibited only when such synthesis was associated with preparation for cell division. RNA and protein synthesis were not inhibited directly *in vivo* unless the cell was in a stage of preparation for mitosis. It was observed that large doses of A-methopterin administered for short periods of time were ineffective and that prolonged periods of administration were required to produce effective inhibitions of nucleic acid synthesis. They reasoned, therefore, that the in-

hibitory effect resulted from depletion of some critical material in the cell—possibly a thymine derivative or a cofactor of which A-methopterin is not a competitive antagonist. Barton & Laird (58) have demonstrated again that cell growth and mitosis are independent but related phenomenon. They report no study of the various stages of mitosis but draw the inference that A-methopterin affects the entrance of a cell into mitosis rather than modifying mitosis during its active process. If this is true, this compound resembles nitrogen-mustard in its antimitotic action. Jacobson (59) has reported that folic acid antagonists such as aminopterin and A-methopterin may arrest mitosis in the interphase. They also observed that, like colchicine, these agents tend to become ineffective on cells exposed to them for a prolonged time. In a more recent publication (60) Jacobson has shown that these antifolic preparations do not affect folic acid itself, as this substance in a ratio as high as 10 to 1 fails to antagonize arrest of cell division by aminopterin, but, in fact, interferes with one of its derivatives, probably the 5-formyl-5:6:7:8-tetrahydro derivative of folic acid. For their studies they used dividing chick fibroblasts and osteoblasts. Jacobson concluded that citrovorum factor (LCF) or a close derivative of it was replaced by aminopterin in the dividing cell. If this were true, it could be reasoned that LCF was probably functioning in the step from metaphase to anaphase. Earlier studies of Jacobson & Webb (61, 62) have shown that aminopterin does not affect the formation of DNA or RNA in the chromosomes in prophase, the disappearance of the nucleolus, or the disruption of the nuclear membrane. However, the metaphase chromosomes were severely affected. Once a cell had passed through metaphase, Jacobson and Webb found no effect on other stages of mitosis or reconstruction of daughter cells with their nucleoli. Jacobson (59) studied the effect of aminopterin and A-methopterin *in vivo* in acute human and mouse leukemia and demonstrated that these compounds under *in vivo* conditions were metaphase inhibitors. Similar effects were obtained by A-methopterin on mouse intestinal epithelium. It was concluded that these agents act by interfering with the parting of the chromosome halves in metaphase and thus prevent the cells from proceeding beyond the stage of metaphase to normal anaphase. Bieseke (63) has investigated the antagonistic effect of 6-mercaptapurine and coenzyme A on mitosis in tissue culture. He was interested in this relationship because of the sulfhydro group which each of these substances bears; moreover, it had been shown by Lasfargues & Wiesendanger (64) that coenzyme A was highly beneficial to tissue cultures in incomplete media.

Bieseke earlier (65) had established that 6-mercaptapurine was a mitotic inhibitor. In cultures of Crocker Mouse Sarcoma 180 and embryonic mouse skin, partial mitotic inhibition produced by 6-mercaptapurine was blocked by coenzyme A. These observations again raise the question of the mechanism of action of carcinolytic compounds such as 6-mercaptapurine, suggesting that any unitary hypothesis of the mechanism of action should be adopted with great caution. In Bieseke's studies, coenzyme A was a more active antagonist of 6-mercaptapurine than other purines, nucleotides, or nucleosides. Although the relationship is not entirely clear it should be re-

called that Mazia's observations indicated that a change in the free —SH content of the cytoplasm is involved in nuclear division. In addition Biesele has shown that 6-mercaptapurine was effective in blocking —SH groups in mitochondria, which led him to suggest that such an action might interfere with the ability of the cell to provide adequate energy for entering mitosis. His observations seem to be an initial bridge between the concepts of Bullock and Swann on the one hand and of Mazia on the other.

Kihlman (66) prepared 20 purine derivatives, most of them by substituting various atoms and groups at the number 8 position in caffeine. He studied the cytological effects on the root meristems of *Allium cepa* using the usual onion test method. The effects observed were, in general, similar to those of radiation. Compounds of one group, including the ethyl-thio-caffeines and the 8-ethers of caffeine, were rapid in action, whereas purines such as caffeine, and 8-fluoro-caffeine, 1,3,7,9-tetramethyluric acid were effective only after prolonged treatment.

It has been shown (67) that the pyrimidines, uracil and thymine, differ in their effects on cell division—uracil producing a fragmentation of the chromosomes in the roots of the onion cells whereas thymine and also cytosine fail to cause any effect although they do decrease the rate of cell division.

A review of the extensive literature of antifolic acids and other carcinolytic purines or pyrimidines is beyond the scope of this review. The reviewer, however, would like to emphasize that the studies on the effect of some of these agents on mitosis seriously challenge concepts that these agents act primarily because they interfere with nucleic acid synthesis. This apparent interference with nucleic acid synthesis may be only a secondary manifestation of a more immediate action on cell division.

Ribonuclease.—It has been suggested that RNA may have some essential function in mitosis (69, 70, 71) as it is associated with the chromosomal tissue in both plants and animals. This idea is strengthened by such indirect evidence as that presented by Kaufmann & Das (68) who have shown that mitotic abnormalities are produced when onion and lily root-tips are treated with ribonuclease. The production of aberrations by ribonuclease was associated with degradation of the ribonucleoproteins.

Amino acids.—The extensive literature on the modification of growth by amino acids and their analogues will not be reviewed here. There have been, however, a few significant recent studies on the mitotic effects of these substances which relate closely to the subject of this review. Biesele & Jacquez (16) have reported on the antimitotic effect of phenylalanine analogues in tissue culture. They found that β -(p-fluorophenyl)-DL-alanine caused a metaphasic delay in cultures of carcinoma T241 and heart. The meta isomer produced similar arrest in tissues of sarcoma 180, an action which was potentiated by adenosine triphosphate. This mitotic inhibition and metaphase arrest could be blocked by L-phenylalanine. Although the mechanism of action of β -(p-fluorophenyl)-DL-alanine is not clear, it is presumed that it is predominantly an effect on cytoplasmic constituents. The observations of Kielier (72), however, suggest that free amino acids in the nucleus may function to regulate the biosynthesis of nucleoprotein.

HORMONES

Adrenal steroids.—The modifications of cell division and development resulting from the direct effects of hormones have not been extensively explored. Such studies have been handicapped by lack of adequate methods of approach. With the improved tissue culture techniques, interest in this problem has been renewed. No attempt will be made here to discuss the action of hormones on biochemical mechanisms unrelated to the subject of this review.

Cortisone and hydrocortisone have been most extensively studied. Siegel *et al.* (73) have administered cortisone to the developing chick embryo. The gross and histological changes observed indicated inhibition of growth and development. These effects could have resulted from either a direct cellular action of cortisone or from an indirect action of cortisone on other endocrine organs. Cortisone and hydrocortisone have been added to cultures of fibroblasts by Holden & Adams (74). Growth inhibition, determined by cell counts, was observed in doses as low as $10\mu\text{g/ml.}$ of substrate. The authors state that growth inhibition was not accompanied by any decrease in mitotic index, nor any change in the distribution of the various phases of mitosis. This is in contrast to the *in vivo* activity of cortisone on mitosis in epithelium of the ears of Wistar rats and CBA and W.L.L. mice (75). The mitotic cycle which normally reached a peak at 2 p.m. each day was completely suppressed. Current investigations in our laboratory (76) indicate that not only does hydrocortisone inhibit the development of HeLa cells grown in tissue culture, but the individual cells have an increased protein content (50 per cent) and increased RNA (60 per cent). The DNA per cell is increased by 13 per cent. The reduction in the number of cells per flask (25 per cent) has been attributed to mitotic inhibition. Although this is probably true, there must be a general slowing down of all mitotic activity because there is, during the first four days, an increase in the mitotic index. There is also a disproportionately large number of dividing cells in metaphase. Cortisone administered to normal mice produces rather striking changes in the parenchymal liver cells (77, 78). There is also recent evidence which indicates that cortisone acetate can modify cell permeability. Setliff *et al.* (79, 80) have shown that this steroid causes increased permeability of ascites tumor cells to water and under appropriate conditions (0° – 5°C. in a K^{++} free media) an increased rate of loss of cell potassium associated with a decreased rate of Na^{++} uptake. Gottlieb (81) using a photographic technique has reported that cortisone when directly added to lymphocytes produces swelling. Lettré (17) has made a successful effort to distinguish between the action of deoxycorticosterone acetate (DOCA) and cortisone in fibroblast tissue cultures. He has found by this technique that cortisone makes sub-threshold doses of colchicine effective whereas DOCA does not. Such reports clearly indicate that adrenal steroids have important *in vitro* actions but, even more important, it appears that we may be approaching the time when the mechanism of action of these steroids will be clarified.

Epinephrine.—Chaudhry *et al.* (82) reinvestigated the effect of epineph-

rine on mitosis and the distribution of mitotic stages in pinnal epidermis of mice. Ten $\mu\text{g.}$ of drug/20 gm. of body weight reduced the number of mitotic figures and modified the percentages of cells in the various stages of mitosis. The percentage of cells in metaphase was 43 in the control and 30 in the treated. The authors, however, were inclined to believe that this was a methodological artifact and that epinephrine acted only as a preprophase inhibitor. Gropp (83), on the other hand, presents evidence from organ cultures of chick embryo that epinephrine inhibits mitosis in metaphase. Adrenochrome was believed to be the active form of the amine. Suvorova (84) has reported that pain or epinephrine injection causes a decline in mitotic activity of the tissues of adult rabbits, cats, mice; adult guinea pigs, however, show no change in mitotic activity with either stimulus. The action of epinephrine should be more carefully evaluated now that we have a better understanding of the biochemical mechanism of many of the actions of this agent (85, 86, 87).

Pituitary hormones.—Leslie in 1952 (88) found that addition of growth hormone to chick embryo fibroblasts *in vitro* produced a slight depression of growth. Fell (89) was, however, unable to show any effect of growth hormone on the growth of chick embryonic limb bones cultivated *in vitro*. Sakae (90) found an increase in mitosis in regenerating forelimbs of the adult newt when the newt was given 2 to 400 $\mu\text{g.}$ of growth hormone. He also observed increased mitosis rates in the ventral fin in the tail of larval bullfrogs on the fourth day after growth hormone administration. Reported and well documented studies of growth hormone on the growth and development of many tissues clearly suggest that stimulation of mitosis *in vivo* must be a common occurrence. Only a few direct reports on mitosis are available, however. Cavallero & Mosca (91) have presented convincing evidence that growth hormone stimulates mitotic activity in the alpha cells of the rat pancreas. Leblond & Carriere (92) found an increase in mitotic activity in the epithelium of the crypts of Lieberkuhn in the duodenum of rats following growth hormone administration to thyroidectomized or hypophysectomized animals. In 1954 Bullough (93) made the interesting observation that growth hormone was an epidermal inhibitor when glucose was employed as substrate. However, when fructose or L-lactate were used as substrate no inhibition was obtained. Bullough observed that this was in agreement with the glucokinase inhibiting effect of growth hormone. This concept was further substantiated by showing that the growth hormone inhibition was, in fact, offset by large doses of insulin. These observations suggest that mitosis is inhibited by limitation of energy production. Because of the large doses of growth hormone required to inhibit mitosis, Bullough suggests that this action may be caused by a metabolic product of the hormone, or a diabetogenic contaminant. Bass & Dunn (94) have shown that the liver of the hypophysectomized rat has a lower percentage of diploid cells and a slight increase in the percentage of binucleate cells. Because growth hormone is so intimately involved in cell growth, it is particularly desirable to extend such limited

studies as have been reported. The lack of appropriate *in vitro* methods has been a serious handicap to the elaboration of the mechanism of action of growth hormone. As recent reports (88, 89, 93) indicate, serious attempts to improve our current or develop new *in vitro* techniques should prove rewarding.

Estrogen.—Bullough (95) has found that estrogens stimulate the rate of mitosis in mouse epidermis. More recently (96) he has shown that the estrogen circulating in the mouse in the stage of pro-oestrus causes an increased rate of mitosis when the ear epidermis is transferred into an *in vitro* culture medium. Ebling (97) was unable to show any effect on the rate of mitosis of estradiol in sebaceous glands implanted in intact, hypophysectomized, or adrenalectomized rats. In 1954 Agrell (98) found that estradiol had a very profound effect on mitosis in sea urchin embryos. The embryonic cleavage was inhibited or delayed in concentrations as low as 2×10^{-6} M. Total inhibition of cellular division was regularly obtained at concentrations of $10-40 \times 10^{-6}$ M. Nuclear division, nonetheless, usually proceeded at these concentrations. However, the production of spindle defects resulted in tri- and tetra-polar spindles. The changes observed closely resembled those produced by colchicine. This estradiol effect was reversible, as normal cell division and the formation of normal spindles occurred when the embryo was returned to fresh sea water. Agrell combined testosterone and estradiol and obtained a synergistic effect. On the basis of further but undocumented experiments with sulfhydryl reagents, he postulated that estrogens may activate cellular sulfhydryl groups.

In contrast to the *in vitro* colchicinelike activity of estrogen on sea urchin eggs, Allen (99) has shown that the injection of 16 μ g. of estradiol benzoate into mice induces an increase in the mitotic activity in the seminal vesicle, in the coagulating gland, in the epidermis of the ear, and in the ventral prostate. The assumption is made that estrogens promote biochemical reactions resulting in a cellular energy level which makes mitosis possible. In contrast to these observations when applied directly to the mouse cornea in concentrations of 1:1000 to 1:10,000, Teti & Langlois (100) observed mitotic inhibition with estradiol. In 1957 Huffman, Jones & Katzberg (101) reported on the antimitotic activity of a series of estrogens. They observed that the histological appearance of the cells was essentially the same as that produced by colchicine. Two screening procedures were employed: (a) the introduction of drug into a tissue culture of heart fibroblasts from chicken embryo; and (b) the addition of drug to a suspension of zebra-fish eggs. Following the treatment of cells in tissue culture with the appropriate estrogen at a concentration of 10^{-7} , metaphase-prophase ratios were recorded. Of four free phenol estrogens, 16-ketotestosterone was the most active with a metaphase-prophase ratio of 72; 16-ketotestradol had a ratio of 57; 16-epitestriol 12.5; diethylstilbestrol 2.3; and colchicine 4.9. Control ratios varied from 3.3 to 4.1. 3-Methyl ethers studied had the following ratios: 16-ketotestosterone 5.7; 16-ketotestradol 105.0; 16-epitestriol 15.0. 3-Ethyl ethers were less active than the parent phenols. Somewhat different results were obtained by the

fish-egg procedure. Stilbestrol was most active, closely followed by estradiol-3, 16 α . It is interesting to observe, in light of certain *in vivo* actions of estrogen, that natural estriol stimulated fibroblastic proliferation of the explants. Unfortunately these workers did not use the mouse ear epidermis test for comparative purposes. It is tempting to suggest that natural estrogens in physiological doses *in vivo* may stimulate mitosis whereas larger doses of natural estrogen or certain synthetic or chemically transformed estrogens may have an opposite effect. On the other hand *in vivo* effects may, in part, be a result of indirect actions secondary to modification of pituitary or adrenal secretions.

MISCELLANEOUS COMPOUNDS

Friedmann *et al.* (102) investigated the antimitotic action of a group of phenolic substances and their derivatives in tissue cultures of chick fibroblasts. The highest activity was shown by hydroquinone and 1,4-naphthohydroquinone. Catechol was inactive. Phosphorylation increased the antimitotic activity of all the phenolic substances tested except resorcinol. Parmentier (103) had previously shown that hydroquinone, 150–175 mg./kg. injected into animals produced abnormalities of the mitotic figures, especially in metaphase, in tissues of the ovaries, uterus, and intestine. Para-benzoquinone applied to onion root tips (104) produced hypertrophy of the cells but no antimitotic action. Loustalot *et al.* (113) have reported that 2-5-bis-ethyleniminohydroquinone has an antimitotic action *in vitro* and *in vivo*. In a series of rat tumors it was carcinostatic in doses that did not affect normal tissues. Ethylene glycol, propylene glycol, and butylene glycol produce mitotic aberrations in both plant and animal tissues (105). Unequal distribution of chromatin and atypical spindles was observed. Seshacher (106) states that carbon tetrachloride produces dissolution of the nuclear membranes of cells from the root tips of several plants. Chromosomal aberrations were also frequently encountered.

Recent studies on urethane and some urethane derivatives (107, 114) show that on the roots of onion bulbs several glycols of urethane have less antimitotic activity than urethane itself. Hydrazine derivatives, however, are more active and the chlorinated derivatives still more effective. Cyclization of urethanes had no effect on the general character of the action exercised on the division of vegetable cells (108) but increased the antimitotic activity. Thio and N-benzyl derivatives were active in low concentrations. Boyland & Koller (109) observed that the mitotic figures were fewer in rat carcinoma when urethane was administered in daily doses of 500 to 1000 mg./kg. Also chromosome fragmentation and anaphase bridges occurred in the dividing cells. Thymine, 50 mg./kg., but not 2,6-diaminopurine or uracil, reduced the frequency of abnormal mitosis and accelerated recovery. In parallel experiments thymine did not affect the number of abnormal mitoses produced by nitrogen mustard.

There have been several reports on the effect of phosphate on the antimitotic properties of known mitotic inhibitors. One of the more interesting

recent observations is that of Friedmann & Simon-Reuss (110) who have shown that toluidhydroquinone and toluquinone develop antimitotic properties apparently by the addition of phosphate at 10^{-3} M to their solutions. Toluidhydroquinone, which itself causes no mitotic inhibition, when combined with phosphate produces a 71 to 84 per cent inhibition of mitosis in tissue culture of chick fibroblasts.

A series of derivatives of aminostilbene has been studied in rats with Walker carcinoma (111). The mitotic disturbances fell into three classes, primary cytotoxic disturbances, nucleotoxic or radiomimetic disturbances, and complex or secondary cytotoxic effects. Moreover, the same agent could produce each of these effects as the dose of the drug administered to the rats varied. Koller (111) and his co-workers concluded that the radiomimetic actions which occurred with low doses could be interpreted as interference with chromosome synthesis. The secondary cytotoxic effects which included fragmentation of chromosomes and gross abnormalities of the mitotic spindle were manifestations of disturbances in general cell metabolism. Avonoff & Graf (112) believe substances such as bromobenzene which interfere with normal mitosis by inhibiting a normal glycolytic pathway should not be classed as mitotic poisons. There is much to be said for their view, for it is evident that any serious disturbance in cell metabolism will eventually be reflected in mitotic aberrations. Druckrey, Danneberg & Schmähl (115) examined approximately 400 compounds including phenols, aromatic amines, and alkaloids and found no strict relationship between structure and blocking action of the cleavage of fertilized eggs of *Paracentrotus lividus*. In general, the most active substances contained *p*-hydroxy or *p*-amino groups. The dose was the primary consideration governing the action on the division of the nucleus or the cytoplasm.

It is not surprising that chelating agents have been studied, in view of the apparent importance of Ca^{++} in the gelation of cytoplasm. McDonald & Kaufman (116) grew roots of onion (*Allium cepa* L) in solutions of ethylenediaminetetra-acetic acid (EDTA) in concentrations of 0.0004 to 0.002 M. Modifications in the form and distribution of the chromosomes of the meristematic cells were observed. Similar results, however, were obtained with 0.002 M CaCl_2 or MgCl_2 . Similar results were reported by Davidson (117), who concluded as did McDonald and Kaufman that the effect of chelating agents would not be attributed to inactivation of particular cations. Kozlik (118) has reported that the antihistamine benzhydrol 2-piperidinoethyl-ether HCl, and N-(*p*-methoxybenzyl-N', N'-dimethyl-N-2-pyridyl) ethylenediamine HCl reduced the rate of mitosis in onion tips, but he observed no harmful effects on the chromosomes. Peters & Lehr (119) treated roots of *V. faba* with Megaphene (N-(3'-dimethylamino)-propyl-3-chlorophenothiazine) and reported a reduction in mitotic rate accompanied by a contraction of the chromosomes. Morphological changes were observed in the cytoplasm and caryoplasm. Oddly enough the ataractic drug, chlorpromazine (120), arrests mitosis of *Allium cepa* in interphase but does not modify mitosis once it is initiated.

THE GIBBERELLINS

Although Japanese botanists have been working for the past 30 years on an amazing group of plant growth substances, only recently have these substances attracted the interest of scientists in Great Britain and the United States. These substances, now known as gibberellins, cause a plant disease long known as bakanae disease. Plants harboring the organism causing this disease grow taller, have larger leaves and longer internodes than unaffected controls. A more complete description of the effects of the gibberellins is found in the excellent review of Stowe & Yamaki (121). Although three different gibberellins have been demonstrated, gibberellic acid has been most thoroughly characterized. Since the gibberellins have qualitatively similar biological action, no distinction will be made between them here.

In 1898 Hori (122) described plant growth of 150 per cent of normal in diseased plants. The increased growth following gibberellin treatment results primarily from stem elongation. This growth is not particularly abnormal, which may in part be a result of the general distribution of the chemical throughout the plant even when applied to one localized area. As might be expected, the treatment is most effective in young plants and becomes less effective as the plant ages (123). Particularly striking has been the increase in stem internodal size unaccompanied by an increase in the number of nodes. It was shown by Kurosawa (124) that individual epidermal and parenchymatous cells of infected rice were longer, with a decreased radial diameter. Although in their early studies Hayashi *et al.* (125) could find no evidence that in rice cell division was affected, Lang (126) concluded that in *Hyoscyamus*, at least under certain conditions, cell division must increase. Moreover, Lona (127), studying a long-day plant, observed that shoot length could not be explained by cell length alone, as the former lengthened more than 30 times whereas the latter increased only four times. Numerous studies (121) to establish more direct evidence of gibberellins on cell division had not been successful until Sachs & Lang (128) found an increased number of cell divisions in the subapical portion of a *Hyoscyamus niger* shoot. Evidence is also presented that gibberellin treatment increases the rate of cell division in the cambial zone. More recently, following the unexpected observation that gibberellin treatment produced considerable growth in the diameter of stems of spur shoots and branches of the apricot one year old or older, Bradley & Crane (129) carefully studied their material microscopically. Sections were taken 3/16 to 1/4 in. below the terminal bud as well as from other segments below this region. A large increase in the treated spurs, both in the radial diameters and the cell counts along the radius of the xylem cylinders, strongly supports the authors' contention that gibberellin stimulates cell division. The radial diameter increased by five times. Similar examinations of the phloem showed no effect of gibberellin on new cell production in this tissue. It appears from these studies that gibberellin stimulates cell division only under properly selected conditions. That this is not a concentration effect is indicated by the expected growth of long shoots and a retardation of bud development on spur shoots. Schroeder & Spector (130)

have added additional evidence that gibberellic acid stimulates cell division. They were able to produce callus formation in citron (*Citrus medica*) explants. Maximum callus formation was produced by five parts per million of the drug. They also showed that this tissue responded to a wider range of gibberellic acid dosage when it was grown in the presence of indoleacetic acid. Haesloop (131) recently has shown in young bean plants that gibberellic acid, at least in part, counteracts the action of maleic hydrazine, a mitotic inhibitor. An additive effect of gibberellins and red light on etiolated bean leaves has been reported by Scott & Liverman (132). Lockhart (133), on the other hand, has reported that gibberellic acid does not promote leaf expansion of dark-grown peas, which may indicate differences between the effect of this agent on the growth mechanisms of beans and peas. Lang (134) has made the interesting observation that these agents induce flower formation in cold-requiring plants and under other conditions of temperature and light which ordinarily do not promote flowering. This leads to the conclusion that gibberellic acid is able to substitute for light in long-day plants. Kahn, Goss & Smith (135) have shown that this chemical also substitutes for red light in the germination of lettuce seed.

The study of the action of gibberellins on the growth of dwarf plants has raised some very intriguing questions concerning the mechanism of action of these agents. Sawada & Kurosawa (136) observed a differential response within one species, which they interpreted as variations in disease resistance. Phinney (137) has reported that in four dwarf maize mutants gibberellin produced growth rates similar to that of treated controls. One mutant showed no response. There was a return to the initial growth rate following cessation of therapy. Since these genetic defects of the dwarf plants were assumed to be single gene defects, it is tempting to reason that gibberellins can substitute for this missing biochemical step. Stowe & Yamaki (121) emphasize that a similar explanation can not hold for the growth of dwarf peas in which the defect is polygenic.

A number of investigators (138, 139, 140) have studied the effect of gibberellin on reproductive development in plants. Gibberellin usually, but not invariably, will substitute for long-day and cold treatments in long-day annuals and biennials. However, this agent will not substitute for any short-day requirement in the initiation of reproductive development. It is the opinion of Vasil (141) that gibberellic acid is probably an auxin as it causes both cell elongation and cell division. Preliminary and poorly documented reports indicate that gibberellic acid has no significant action on animal cells either *in vivo* or in tissue culture.

SUMMARY

When one considers the wide variety of chemical substances that modify cell division, it becomes immediately apparent that: (a) either more than one biochemical mechanism is involved; or (b) that there is a nonspecific action on some single function or structure of the cell which in turn is reflected in

mitotic irregularities. Such a structure could be the cell membrane, and the function might be permeability to essential metabolites. Our knowledge of the mechanisms available indicates that both of the above possibilities must be seriously entertained. That interference with functional —SH, carboxyl, or phosphate groups, with normal H-bonding, with cytoplasmic Ca^{++} concentration, with cytoplasmic gelation, or with metabolite absorption of transfer may be involved is clearly evident. The relative importance or the interrelationship of these, however, is by no means clear. Such an important problem deserves much more attention than it has received. Investigation of the mechanism of mitosis and the action of antimitotic agents should be particularly rewarding because characteristic physical changes in the cell can be correlated with biochemical phenomena. Relatively few studies have been made on the correlation of the biochemistry of the cell and nucleus during mitosis in cultures of cells with synchronized stages of mitosis. Perhaps greater progress would be made if more attention were given to the study of the mechanism of drug action because much is frequently learned by distorting normal physiological processes. It would be particularly valuable to have each drug considered in broader perspective, so that its effect on the various parameters of cellular and nuclear biochemistry could be defined more precisely. It would seem reasonable to assume that different mechanisms of drug action must be evoked to explain arrest of mitosis at metaphase and arrest of mitosis in interphase or preprophase. It is also possible that certain agents modify cytoplasmic and other nuclear biochemistry. Other agents may, in turn, modify the normal functioning of the cell or nuclear membrane. The reviewer had hoped that he might be able to categorize the action of antimitotic chemicals. Although certain agents appear to have reasonably specific actions, so many contradictory observations have been recorded that no useful purpose would be served by any necessarily arbitrary classification. Normal mitosis may be dependent on such a complex series of reactions that its modification is comparable to the effects of drugs on cell growth where interference with any one of many biochemical pathways modifies the normal growth pattern.

LITERATURE CITED

1. Dustin, A. P., *Compt. rend. assoc. anat.*, **33**, 204 (1938)
2. Levine, M., *Ann. N. Y. Acad. Sci.*, **51**, 1365 (1951)
3. Brachet, J., *Biochemical Cytology* (Academic Press, Inc., New York, N. Y., 516 pp., 1957)
4. Ludford, R. J., *Arch. exptl. Zellforsch. Gewebesücht.*, **18**, 411 (1936)
5. Beams, H. W., and Evans, T. C., *Biol.*, **79**, 188 (1940)
6. Wilbur, K. M., *Proc. Soc. Exptl. Biol. Med.*, **45**, 696 (1940)
7. Dustin, A. P., *Bull. classe sci. Acad. roy. Belg.*, (5), **14**, 487 (1954)
8. Mazia, D., *Symposia Soc. Exptl. Biol.*, **9**, 355 (1955)
9. Mazia, D., *Advances in Biol. and Med. Phys.*, **4**, 70 (1956)
10. Mazia, D., *Enzymes: Units of Biological Structure and Function*, 261 (Gaebler, O. H., Ed., Academic Press, Inc., New York, N. Y., 642 pp., 1956)

11. Levan, A., *Hereditas*, **40**, 1 (1954)
12. Dustin, P., *Nature*, **159**, 794 (1947)
13. Friedmann, E., Marrian, D. H., and Simon-Reuss, I., *Brit. J. Pharmacol.*, **3**, 335 (1948)
14. Friedmann, E., Marrian, D. H., and Simon-Reuss, I., *Brit. J. Pharmacol.*, **3**, 263 (1949)
15. Lettré, H., *Naturwissenschaften*, **38**, 490 (1951)
16. Biesele, J. J., and Jacquez, J. A., *Ann. N. Y. Acad. Sci.*, **58**, 1276 (1954)
17. Lettré, H., *Cancer Research*, **12**, 847 (1952)
18. Hughes, A. F., *J. Microscop. Sci.*, **91**, 251 (1950)
19. Swann, M. M., *Cancer Research*, **17**, 727 (1957)
20. Gross, P. R., *Trans. N. Y. Acad. Sci.*, **11**, 20, 154 (1957)
21. Mazia, D., *Symposium on Chemical Basis of Heredity* (McElroy, W. D., and Glass, B. B., Eds., Johns Hopkins Press, Baltimore, Md., 848 pp., 1957)
22. Mazia, D., and Roslansky, J. D., *Protoplasma*, **46**, 528 (1952)
23. Nishimura, E. T., and Baum, J. H., *Arch. Pathol.*, **63**, 513 (1957)
24. Nishimura, E. T., *Arch. Pathol.*, **63**, 352 (1957)
25. Padawer, J., and Gordon, A. S., *Proc. Soc. Exptl. Biol. Med.*, **88**, 522 (1955)
26. Moritz, O., and Heldt, L., *Ber. deut. botan. Ges.*, **67**, 160 (1954)
27. Gati, E., Inke, G., and Palkovits, M., *Acta Morphol. Acad. Sci. Hung.*, **7**, 335 (1957)
28. Gropp, H., *Z. Krebsforsch.*, **60**, 9 (1954)
29. Resegotti, L., and von Euler, H., *Tumori*, **41**, 168 (1955)
30. Truhaut, R., and Deysson, G., *Ann. pharm. franç.*, **14**, 416 (1956)
31. Deysson, G., and Truhaut, R., *Ann. pharm. franç.*, **14**, 434 (1956)
32. Lenti, G., and Tortarolo, E., *Minerva Med.*, **1**, 21 (1957)
33. Linskens, H. F., and Wulf, W., *Naturwissenschaften*, **40**, 487 (1953)
34. Muhlemann, H. R., Marthaler, T. M., Rateitschak, K., and Loustalot, P., *Bull. Schweiz. Akad. med. Wiss.*, **12**, No. 2, 163 (1956)
35. Benitez, H. H., Murray, M. R., and Chargaff, E., *Ann. N. Y. Acad. Sci.*, **58**, 1288 (1954)
36. Heilbrunn, L. V., *Outline of General Physiology* (W. B. Saunders Co., Philadelphia, Pa., 327 pp., 1952)
37. Hultin, T., *Exptl. Cell Research*, **1**, 159 (1950)
38. Hultin, T., *Exptl. Cell Research*, **1**, 272 (1950)
39. Gross, P. R., *Biol. Bull.*, **103**, 293 (1952)
40. Gross, P. R., *J. Cellular Comp. Physiol.*, **47**, 429 (1956)
41. Schneider, W. C., *J. Biol. Chem.*, **166**, 595 (1946)
42. Price, C. C., *Ann. N. Y. Acad. Sci.*, **68**, 663 (1958)
43. Ross, W. C. J., *Ann. N. Y. Acad. Sci.*, **68**, 669 (1958)
44. Alexander, P., *Advances in Cancer Research*, **2**, 2 (1954)
45. Stacey, K. A., Cobb, M., Cousens, S. F., and Alexander, P., *Ann. N. Y. Acad. Sci.*, **68**, 682 (1958)
46. Alexander, P., *Nature*, **169**, 226 (1952)
47. Kay, E. R. H., Simmons, N. S., and Dounce, A. L., *J. Am. Chem. Soc.*, **74**, 1724 (1952)
48. Kay, E. R. H., and Dounce, A. L., *J. Am. Chem. Soc.*, **75**, 4041 (1952)
49. Hutchens, J. O., and Podolsky, B., *J. Cellular Comp. Physiol.*, **43**, 205 (1954)
50. Loveless, L. E., Spoerl, E., and Weisman, T. H., *J. Bacteriol.*, **68**, 637 (1954)
51. Katchman, B. J., Spoerl, E., and Smith, H. E., *Science*, **121**, 97 (1955)
52. Powers, E. L., and Pomeroy, J. H., *Ann. N. Y. Acad. Sci.*, **68**, 702 (1958)

53. Powers, E. L., and Ehret, C. F., *Proc. Intern. Conf. Peaceful Uses Atomic Energy*, **11**, 266 (1956)
54. Roberts, J. J., *Ann. N. Y. Acad. Sci.*, **68**, 722 (1958)
55. Alexander, P., and Stacey, K. A., *Ann. N. Y. Acad. Sci.*, **68**, 1225 (1958)
56. Nichol, C. A., and Welch, A. D., *Proc. Soc. Exptl. Biol. Med.*, **74**, 403 (1950)
57. Jukes, T. H., *Federation Proc.*, **12**, 633 (1953)
58. Barton, A. D., and Laird, A. K., *J. Biol. Chem.*, **227**, 795 (1957)
59. Jacobson, W., *J. Physiol. (London)*, **123**, 603 (1954)
60. Jacobson, W., *J. Physiol. (London)*, **123**, 618 (1954)
61. Jacobson, W., and Webb, M., *Exptl. Cell Research*, **3**, 163 (1952b)
62. Jacobson, W., and Webb, M., *Endeavour*, **11**, 200 (1952b)
63. Bieseke, J. J., *J. Biophys. Biochem. Cytol.*, **1**, 119 (1955)
64. Lasfargues, E., and Wiesendanger, S., *Compt. rend. soc. biol.*, **147**, 978 (1953)
65. Bieseke, J. J., *Ann. N. Y. Acad. Sci.*, **60**, 228 (1954)
66. Kihlman, B., *Proc. Intern. Botan. Congr., 7th Cong. Stockholm*, **7**, 212 (1950)
67. Deysson, M., *Compt. rend. hebdomadaires acad. sci.*, **238**, 145 (1954)
68. Kaufmann, B. P., and Das, N. K., *Proc. Natl. Acad. Sci.*, **40**, 1052 (1954)
69. Ledoux, L., and Revell, S. H., *Biochim. et Biophys. Acta*, **18**, 416 (1955)
70. Brody, S., *Biochim. et Biophys. Acta*, **24**, 502 (1957)
71. Chevreumont, M., and Chevreumont-Comhaire, S., *Compt. rend. soc. belge biol.*, **149**, 1525 (1955)
72. Kieller, J., *Acta Pathol. Microbiol. Scand.*, **34**, 1 (1954)
73. Siegel, B. V., Smith, M. J., and Gerstl, B., *Arch. Pathol.*, **63**, 562 (1957)
74. Holden, M., and Adams, L. B., *Proc. Soc. Exptl. Biol.*, **95**, 364 (1957)
75. Ghadially, F. N., and Green, H. N., *Brit. J. Exptl. Pathol.*, **38**, 100 (1957)
76. Bass, A. D., Cheatham, C. (Unpublished data)
77. Lowe, C. V., and Rand, R. N., *J. Biophys. Biochem. Cytol.*, **2**, 331 (1956)
78. Dunn, C. E., Bass, A. D., McArdle, A. H., *Exptl. Cell Research*, **14**, 23 (1958)
79. Setliff, J. A., Bass, A. D., Cheatham, C. (Unpublished data)
80. Setliff, J. A., Bass, A. D., Cheatham, C., *Federation Proc.*, **17**, 411 (1958)
81. Gottlieb, L. S., and Dougherty, T. F., *Proc. Am. Assoc. Cancer Research*, **2**, No. 4, 343 (1958)
82. Chaudhry, A. P., Halberg, F., and Bittner, J. J., *J. Appl. Physiol.*, **9**, 265 (1956)
83. Gropp, A., *Z. Krebsforsch.*, **60**, 52 (1954)
84. Suvorova, L. V., *Doklady Akad. Nauk S.S.S.R.*, **110**, 293 (1956)
85. Sutherland, E. W., and Wosilait, W. D., *J. Biol. Chem.*, **218**, 459 (1956)
86. Wosilait, W. D., and Sutherland, E. W., *J. Biol. Chem.*, **218**, 469 (1956)
87. Rall, T. W., Sutherland, E. W., and Wosilait, W. D., *J. Biol. Chem.*, **218**, 483 (1956)
88. Leslie, I., *Biochem. J.*, **52**, xxi (1952)
89. Fell, H. B., *The Hypophyseal Growth Hormone, Nature and Actions*, **138-48** (McGraw-Hill Book Co., Inc., New York, N. Y., 576 pp., 1955)
90. Sakae, I., *Endocrinol. Japon.*, **3**, 236 (1956)
91. Cavallero, C., and Mosca, L., *J. Pathol. Bacteriol.*, **66**, 147 (1953)
92. Leblond, C. P., and Carriere, R., *Endocrinology*, **56**, 261 (1955)
93. Bullough, W. S., *Exptl. Cell Research*, **7**, 186 (1954)
94. Bass, A. D., and Dunn, C. E., *Proc. Soc. Exptl. Biol. Med.*, **96**, 175 (1957)
95. Bullough, W. S., *Biol. Revs. Cambridge Phil. Soc.*, **27**, 133 (1952)
96. Bullough, W. S., *Exptl. Cell Research*, **7**, 176 (1954)
97. Ebling, F. J., *J. Endocrinol.*, **12**, 38 (1955)

98. Agrell, I., *Nature*, **173**, 172 (1954)
99. Allen, J. M., *Exptl. Cell Research*, **10**, 523 (1956)
100. Teti, M., and Langlois, M., *Compt. rend. soc. biol.*, **148**, 1031 (1954)
101. Huffman, M. N., Jones, R. W., and Katzberg, A. A., *Cancer*, **10**, 707 (1957)
102. Friedmann, E., Marrian, D. H., and Simon-Reuss, I., *Biochim. et Biophys. Acta*, **13**, 260 (1954)
103. Parmentier, R., *Compt. rend. soc. biol.*, **147**, 935 (1953)
104. Garrigues, R., and Buu-Hoi, N. P., *Compt. rend. soc. biol.*, **147**, 963 (1953)
105. Hasek, M., and Holeckova, E., *Biol. listy*, **30**, 83 (1949)
106. Seshacher, B. R., and Nambiar, P. K., *Nature*, **176**, 796 (1955)
107. Truhaut, R., and Deysson, G., *Compt. rend.*, **244**, 2189 (1957)
108. Truhaut, R., and Deysson, G., *Compt. rend.*, **244**, 2257 (1957)
109. Boyland, E., and Koller, P. C., *Brit. J. Cancer*, **8**, 677 (1954)
110. Friedmann, E., and Simon-Reuss, I., *Experientia*, **10**, 494 (1954)
111. Koller, P. C., *J. Natl. Cancer Inst.*, **15**, 1237 (1955)
112. Avonoff, S., and Graf, G. E., *Nature*, **172**, 1043 (1953)
113. Loustalot, P., Schar, B., and Meier, R., *Experientia*, **11**, 186 (1955)
114. Truhaut, R., and Deysson, G., *Ann. pharm. franç.*, **15**, 324 (1957)
115. Druckrey, H., Danneberg, P., and Schmähel, D., *Pubbl. staz. zool. Napoli.*, **24**, 247 (1953)
116. McDonald, M. R., and Kaufman, B. P., *Exptl. Cell Research*, **12**, 415 (1957)
117. Davidson, D., *Exptl. Cell Research*, **14**, 329 (1958)
118. Kozlik, V., *Pharmazie*, **9**, 547 (1954)
119. Peters, K., and Lehr, H., *Arch. exptl. Pathol. Pharmacol.*, Bd. **221**, S. 365 (1954)
120. Deysson, G., Decourt, P., and Anguera, G., *Rev. pathol. gén. comparée*, **54**, 868 (1954)
121. Stowe, B. B., and Yamaki, Toshio, *Ann. Rev. Plant Physiol.*, **8**, 181 (1957)
122. Hori, S., *Mem. Agr. Research Sta. (Tokyo)*, **12**(1), 110 (1898)
123. Marth, P. C., Audia, W. V., and Mitchell, J. W., *Plant Physiol.*, **31**, Suppl. xliii (1956)
124. Kurosawa, E., *Trans. Natl. Hist. Soc. Formosa*, **18**(97), 230 (1928)
125. Hayashi, T., Takijima, Y., and Murakami, Y., *Nippon Nôgei-kagaku Kaishi*, **27**(10), 672 (1953)
126. Lang, A., *Naturwissenschaften*, **43**, 257 (1956)
127. Lona, F., *Nuovo giorn. botan. ital.*, **63**(1), 61 (1956)
128. Sachs, R. M., and Lang, A., *Science*, **125**, 1144 (1957)
129. Bradley, M. V., and Crane, J. C., *Science*, **126**, 972 (1957)
130. Schroeder, C. A., and Spector, C., *Science*, **126**, 701 (1957)
131. Haesloop, J. G., Jr., *J. Elisha Mitchell Sci. Soc.*, **73**, No. 2 (Nov. 1957)
132. Scott, R. A., Liverman, J. L., *Science*, **126**, 122 (1957)
133. Lockhart, J. A., *Proc. Natl. Acad. Sci. U. S.*, **42**, 841 (1956)
134. Lang, A., *Naturwissenschaften*, **43**, 284 (1956)
135. Kahn, A., Goss, J. A., Smith, D. E., *Science*, **125**, 645 (1957)
136. Sawada, K., and Kurosawa, E., *Taiwan Sotokufu Chuo Kenkyusho Nogyobu Iho*, No. **50**, 1 (1927)
137. Phinney, B. O., *Proc. Natl. Acad. Sci. U. S.*, **42**, 185 (1956)
138. Lang, A., *Proc. Natl. Acad. Sci. U. S.*, **43**, 709 (1957)
139. Bunsow, R., Harder, R., *Naturwissenschaften*, **43**, 479 (1956)
140. Greulach, V. A., Haesloop, J. G., *Science*, **127**, 646 (1958)
141. Vasil, I. K., *Science*, **126**, 1295 (1957)

CONNECTIVE AND SUPPORTING TISSUES: BONE¹

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The physiology of connective and supporting tissues has been reviewed in recent years (1, 2). Not since 1950 (3), however, has bone received special attention. In the meantime, interest in bone and corresponding investigative effort in its ultrastructure, biochemistry, and physiology have gone forward at an accelerated pace. The scope of this review has had to be severely limited, because of the disproportion between allotted space and the large number of contributions. First consideration has been given to bone, as a highly specialized connective and supporting tissue; recent contributions to the physiology of other connective tissues have been referred to, especially when the advances they record are applicable also to bone.

A number of important monographs, reviews, and symposia have appeared during the past few years. They cover a wide range of subject matter in relation to bone and other connective tissues, including reviews of the older literature. Some are of broad, general interest (4 to 16); other more specialized contributions will be referred to under appropriate topics. This review attempts to link current knowledge of the physiology of bone with that recorded by Dallemagne (3) in 1950; of the publications consulted, preference has been given to those of recent date and to those with references to earlier contributions.

THE STRUCTURE AND FUNCTION OF BONE

While the intimate structure of bone, as observed with the light microscope, has been well described for many years, the exploitation of new biophysical techniques has added much information concerning the details of fine structure and has increased knowledge of the relation of structure to function. Reviews and monographs are available [Engström (6)], (17 to 20). Special emphasis on the interrelations of the chief components of bone is necessary for an understanding of function.

Ultrastructure of bone.—The terms fine structure and ultrastructure, as well as submicroscopic, have been used to indicate particles or structures not resolvable by the light microscope. High-resolution electron microscopy refers specifically to dimensions of 30 Å or less. Such techniques as x-ray diffraction and electron diffraction give indirect information concerning ultrastructure and organization at the submicroscopic level. Microradiography, combined with autoradiography, as applied to bone during the period covered by this review, has extended the possibilities of direct observation of very small structures (21, 22). The polarizing microscope has

¹ The survey of literature pertaining to this review was concluded in June, 1958.

long been a classical tool for research in ultrastructure of calcified tissues, and the interference microscope has afforded information in agreement with that obtained by microradiography (17). A recent addition, that of x-ray absorptiometry, permits the determination of very small concentrations of strontium in the presence of calcium (23). The investigator has now at his disposal an armamentarium of techniques, the potentialities of which have by no means been exhausted. The simultaneous use of two or more techniques, rather than strict adherence to one, is proving especially fruitful in elucidating the fine structure of bone and its relations to function.

THE ORGANIC MATRIX OF BONE

The organic matrix of bone, accounting for 35 per cent or more of the dry, fat-free weight of bone, is described in two parts: (a) collagen fibers, which make up about 95 per cent of the organic portion by weight; and (b) the ground substance, filling the spaces between the collagen fibers and the crystals of bone mineral. This has been described as amorphous; recent electron microscope observations indicate that it has an organization and ultrastructure of its own (24, 25).

Collagen.—The collagen of bone has been little studied, but it has characteristics in common with those of other forms of connective tissue (26, 27). It is believed that the unit building block of collagen, called tropocollagen, is secreted by osteoblasts in soluble form and that the macromolecules aggregate in insoluble form as fibrils (28). The usual characteristics of these fibrils are: (a) a distinctive x-ray pattern; (b) an unusual amino acid composition; and (c) an axial periodicity of 640 Å with detailed intraperiod fine structure. Electron micrographs of bone have revealed that the bands characteristic of the collagen fibers tend to be in register between adjacent fibers (29). The collagen fibers of bone, except when resorbed and replaced in the remodeling process, are subject to a very slow turnover (30, 31, 32).

Ground substance.—A distinction is made between the structural components of the ground substance and the chemical constituents which may or may not contribute to its structure [Meyer (6)]. The ground substance varies in consistency from that of the interstitial fluid to that of the basement membranes. It is coextensive with both; they represent its fluid and condensed portions respectively (33). Chemically the ground substance is characterized by its content of polysaccharides containing hexosamines, or amino sugars, and by its staining reactions. In recent years the chemistry of the mucopolysaccharides of connective tissue, including bone, has received major attention (2, 34). Because of the mineral content of bone, the structure and organization of its ground substance do not lend themselves readily to study. In the case of rat-tail tendon, the nonfibrillar ground substance has been described, from electron microscopic observations, as consisting of two phases: (a) water-rich, protein-poor vacuoles; and (b) their water-poor, protein-rich walls (24). The two phases are believed to be in equilibrium. It is also suggested that collagen fibrils are formed in the walls of the vacuoles; i.e., in the dense phase of the ground substance.

CHEMISTRY AND CRYSTAL STRUCTURE OF BONE MINERAL

Despite intensive efforts over the past century and despite the great advances made in recent years, the chemistry and ultrastructure of the bone mineral remain fertile fields for inquiry. It is now firmly established (11) by chemical analysis, x-ray diffraction, and by other methods that the crystalline material found in bone belongs to the apatite series of minerals and that it corresponds closely in composition and crystal structure to hydroxyapatite: $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$. There are, however, differences in interpretation of the evidence now available (35 to 37). While the bone mineral contains considerable amounts of carbonate and citrate, current opinion is to the effect that these ions are related to the surfaces of the crystals of hydroxyapatite and that they are not part of the lattice structure (11). Other ions, such as Na^+ , K^+ , Mg^{++} , Cl^- , and F^- , are found in the mineral, being deposited during crystal growth; the exact composition of the bone mineral is in part determined by the diet and by the composition of the fluid from which it is formed (38). The molar ratio Ca/P, theoretically 1.66, varies from 1.3 to 2.0 in a series of solid calcium phosphates which give the characteristic apatite lattice pattern on x-ray diffraction; this variation is attributed to the extremely small size of the crystals and to the correspondingly large influence of the contributions of their surface ions to the analysis of the total mineral (39).

One school still holds tenaciously to the view that the bone mineral is essentially hydrated calcium triphosphate (alpha tricalcium phosphate), to which is assigned the formula: $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{H}_2(\text{OH})_2$. In support of this thesis, data from ion exchange reactions are reported (40) and interpreted to indicate that this compound is unstable, that its instability is responsible for its reactivity in the living organism, and that on isolation from bone it is transformed into the stable hydroxyapatite.

It is convenient to regard the unit of the mineral of bone, at the ultrastructural level, as a crystal complex, each such complex being based upon a microcrystal of colloidal dimensions, only a few hundred A in length and with a thickness of 20 to 50 A, or that of a few unit cells. The crystals have been described, from electron microscopic and x-ray diffraction evidence, as hexagonal tablets (29), and as needle- or rod-shaped (41 to 44). Each crystal complex includes the crystal itself, surrounded by a series of layers of water forming the hydration shell (39). Within the shell are hydrated ions, bound to the surfaces of the crystals, and other ions in solution, readily exchangeable with the intercellular fluids of the bone. The crystal complex, as a whole, participates in the dynamics of the transfer of ions between bone and blood.

The portion of the calcium of the bone mineral commonly designated as exchangeable constitutes a small fraction of the total skeletal calcium. The exchangeable fraction decreases with age, and is currently estimated at a total of approximately 5 gm. in adult man (45).

Of current interest is the interrelation of the collagen fibers, with their cross-banding, and the mineral of bone. Small particles approximately 100 A

in diameter have been described between the bands in the earliest stage of calcification (46). In fully calcified bone the crystals, oriented in the fiber axis, tend to form bands around the collagen fibers, leaving gaps between the bands (29); these gaps correspond to the spacing of the collagen fibers between the periodic cross bands. It is now stated that there are also crystals of mineral, situated within the collagen fibers (47).

HISTOPHYSIOLOGY OF BONE

For the purposes of this review it is desirable to consider the structural and chemical elements of bone as an integrated system, within and on the surfaces of which the functions of osseous tissue are carried out. The bone as a whole participates in growth, remodeling, and repair, to which attention is directed individually. For the intimate metabolic activity of bone, including transfers of ions between bone and blood, and the nourishment and maintenance of bone as a living tissue, the components of the system, organic and inorganic, are all interrelated.

Ground substance.—The ground substance of bone supplies the internal environment for the crystals of bone mineral, for the collagen fibers, and for the cells incorporated within bone (osteocytes). It includes the mucopolysaccharides of bone, which are related to similar substances in other connective tissues and which presumably are the intermediate or end products of the metabolism of the bone cells. It is believed that the ground substance plays an important but as yet poorly defined part in the seeding of crystals of bone mineral (48). The current interest in the chemistry of the mucopolysaccharides (49) reflects a belief that the ground substance has a much more important part in the metabolism and intimate physiology of bone, as well as of other connective tissues, than had previously been recognized. Special attention has been given to the intercellular components of connective tissue, including fibers and ground substance, in a recent comprehensive review (50).

Osteoblasts and osteocytes.—The three types of cells that characterize bone—osteoblasts, osteocytes, and osteoclasts—are closely interrelated and are readily transformed one into the other, in both structure and function (51). The osteoblast has been given consideration in an extensive review [Pritchard (5, Chap. 7)]. Beyond recognition that osteocytes are the living elements of bone, some attention has been given to the manner in which they function (52). It is here suggested that this function is concerned chiefly with maintenance of the integrity of the organic matrix of bone and that the metabolic activity of the osteocytes is reflected in the composition and chemical reactions of the ground substance. The osteocytes may influence both the metabolism and the composition of the intercellular substance (53).

Resorption of bone; the osteoclast.—A recent review is basic to consideration of the osteoclast and its relation to resorption of bone [Hancox (5, Chap. 8)]. Resorption has been reviewed (54) and it has been suggested that the solution of mineral in the resorption of bone is accomplished by

chelation (54, 55). Recent studies of the morphology and histochemistry of osteoclasts (56) and of their bone-destroying function (57) have appeared. In the latter, special attention is given to the functional significance of Koelliker's brush border. Whereas earlier microscopic studies appeared to have ruled out phagocytosis of bone mineral as a possible mechanism in osteoclastic resorption, recent electron microscope observations have demonstrated crystals in sections through osteoclasts (44, 58). Concentration of plutonium in osteoclasts has also been reported (59).

Growth of bone.—A review of bone growth in health and disease has been published (60). There have been important studies of morphogenesis, both at the microscopic (61, 62) and submicroscopic levels (28). The three-dimensional anatomy of Haversian systems has been described in detail (63).

Repair of bone.—In recent years attention has been centered on transplants or grafts of osseous tissue, largely in connection with the healing of fractures or the filling in of defects in bone structure. There is now general agreement that transplants of fresh autogenous cancellous bone or of bone marrow may survive in the host bed and produce new bone (64 to 67). There is less evidence for the survival and growth of transplants of homogenous bone (68) and little or none for the survival of heterogenous grafts. It is recognized, however, that implants of frozen-dried, devitalized, or preserved heterogenous bone may lead to production of new bone from the host bed, by induction (69). Similar results are reported for implants of the decalcified organic matter of bone (70) and of bone mineral, prepared by removal of organic matter by solution in ethylene diamine (71). A number of biophysical techniques have been applied to the study of transplants and implants of bone (72). The idea, which had apparently been laid to rest, of an osteogenic inductor factor isolated in solution from bone has recently been revived (73) with reports of induction of new bone formation by implants of Gelfoam impregnated with an extract of bone paste (74). On the other hand, tissue transfers *in vivo*, separated from the cells of the host by Millepore membranes have given no evidence of the passage of any osteogenic inductor substance (75).

Remodeling of bone.—By a combination of histologic study, microradiographs, and autoradiographs, all on the same sections of bone (20, 76), new light has been shed on the mechanisms and functional significance of the remodeling of compact bone. Such studies have been supplemented by the use of lead as a marker (77); this is fixed in osteons in the course of osteogenesis and allows evaluation of the speed of their formation. The formation of resorption cavities or tunnels, described many years ago, and their subsequent reconstruction into new Haversian systems, or osteons, take on new significance (78). An average resorption cavity in a dog requires roughly three weeks to form (79). The tunnel then becomes lined with osteoblasts and is filled in with concentric layers or lamellae of bone, within which are incorporated the lacunae and canaliculi characteristic of the haversian system. The building of the osteon, including partial calcification of the organic

matrix, requires some 6 to 12 weeks. Primary mineralization of the matrix, to about 70 per cent of the final content, occurs rapidly during and immediately after the deposition of new layers of organic material; completion of secondary mineralization, to maximum density, takes much longer and has been found to be incomplete at 18 weeks (77).

Remodeling of bone, formerly believed to be determined by the weight-bearing and other functions of the skeleton as a whole, is now seen to have an important metabolic function in the maintenance of a continuous supply of reactive bone, available for the transfer of calcium and phosphate between bone and blood (80). The most reactive bone, both for deposition and exchange of mineral, is demonstrable in autoradiograms as the youngest bone forming the lining of osteons under construction (78). Some uptake of radioisotopes also occurs in old compact bone, and this may even exceed, in total amount, that taken up by the new osteons (79), but this diffuse uptake is much less per unit of mass than that in new bone matrix. It is reported by others that uptake of radiocalcium in fully mineralized osteons is absent or negligible, either in an absolute sense or relative to that taken up by newly deposited matrix (11).

THE DYNAMICS OF THE PHYSIOLOGY OF BONE

The chemical dynamics of the bone mineral has recently been reviewed in detail (11). There are still many gaps in the understanding of the physico-chemical forces as well as of the biologic influences in bone as a living tissue. While much effort has been directed to the mechanisms concerned with the apposition of bone mineral and with the transfer of ions between bone and blood, the interrelations between the inorganic and organic elements of osseous tissue are of equal importance and are also under close scrutiny.

It is desirable at the outset to emphasize the difference between ossification and calcification. Ossification is the formation of bone tissue; calcification is the deposition of a calcium-containing mineral in any organic material. Ossification may be understood to include calcification but the two terms are not synonymous.

THE MECHANISM OF CALCIFICATION

A further differentiation must be made between calcification and precipitation. Calcification requires nucleation followed by crystal growth, and it has been shown that the formation of crystals of hydroxyapatite is not predictable by application of any simple solubility product constant (81). Moreover, while the only solid phase formed by calcium and phosphate in the physiologic pH range is hydroxyapatite, the initial aggregation of calcium and phosphate ions is as CaHPO_4 . And while the interstitial fluids of bone must be assumed to be in equilibrium with the solid phase, normal serum is now described as undersaturated with respect to CaHPO_4 , but supersaturated with respect to hydroxyapatite (11). This paradoxical situation has rendered vastly more difficult an adequate understanding of the

chemical characteristics of the bone mineral, and of its behavior in the living organism.

Since the body fluids are supersaturated with respect to the final product—hydroxyapatite—it follows that crystal growth may continue, once crystal seeding has occurred. The problem, only partially solved, is to show how crystal seeding can be initiated from serum undersaturated with CaHPO_4 . This has been attempted, with some success, by postulating a biologic process which controls the initiation of crystal nucleation. Concurrently, the alternative explanation that initiation of calcification requires spontaneous crystal formation induced by local increase in concentration of phosphate ions to the point of supersaturation with CaHPO_4 has been shown to be untenable (11).

Crystal seeding or nucleation.—The critical stage in calcification is its initiation; once crystal nuclei are formed, crystal growth follows the general pattern seen in crystallization induced in aqueous solutions. Attempts to elucidate the mechanism of the initiation of calcification go back for many years [Dixon and Perkins (5, Chap. 10)] and are still continuing. Much of the work has been done *in vitro* on cartilage, some of it noncalcifiable, and its applicability to the conditions in bone *in vivo* is at times doubtful.

Among the early proposals was that calcium was bound by cartilage and that subsequently phosphate combined with the calcium to form the bone mineral. This was followed shortly by the reverse proposal, i.e., that phosphate was the main ion bound to the organic material of cartilage and that calcium then combined with the bound phosphate. These proposals still persist in the modern literature.

Because of the close physical relationship of the mineral with the collagen fibers of bone, which includes orientation of the crystals in the long axes of the fibers and localization of the crystals in relation to the cross banding of the fibers (82), the current tendency is to assign a special function in the initiation of calcification, or seeding of the crystals, to collagen. This has been suggested in at least four forms (39): (a) that the collagen fibers possess the chemical property of inducing the production of crystal nuclei, perhaps through the presence on the collagen molecule of a phosphorylated amino sugar (83); (b) that, particularly in cartilage, the protein is activated by the enzymatic transfer of a pyrophosphate group from ATP to collagen (84, 85); (c) that the active nucleation center in cartilage involves a complex between collagen and chondroitin sulfate (48); and (d) that the mechanism of nucleation involves a specific stereochemical configuration resulting from a particular state of aggregation of collagen macromolecules (86). All of these proposals regard the protein component—collagen, and possibly also the protein of the ground substance—as part of the mechanism, and all imply that a protein template is responsible for a special spatial arrangement which lends itself to the nucleation and growth of the crystals of hydroxyapatite. None of these proposals, however, accounts satisfactorily for the fact that calcification occurs in some locations and not in others although both may be rich in collagen, ground substance, and phosphatase. The explanation of-

ferred some years ago (87) to the effect that the property of calcifiability depends upon the state of polymerization, or aggregation, of the ground substance, while attractive, remains unsubstantiated.

Other explanations have been offered, some of which are variants of those given above. Inhibition of calcification of rachitic cartilage *in vitro* by metachromatic and other basic dyes has led to the inference that chondroitin sulfate ester participates in normal calcification (88). It is also proposed that calcium ions are bound with one of their valences to chondroitin sulfate, with the other bound to pyrophosphate, which in turn is bound to the protein matrix (89). This hypothesis also assumes a change in the aggregation states of protein and mucopolysaccharide molecules, leading to formation of the crystals of bone mineral. The idea that collagen, itself an organic crystalline material, acts as a template for crystal seeding, has been expanded by reference to the phenomenon of epitaxy but without reaching a final conclusion in this respect (11).

Crystal growth.—Once crystal seeding or nucleation has occurred, growth of the crystals follows but only to colloidal dimensions. Even under extreme conditions of temperature and pressure, crystals of hydroxyapatite not larger than 0.1 mm. in length have been obtained (90). From the preceding section it will be seen that crystals add to their mass in the presence of serum supersaturated with hydroxyapatite but undersaturated with CaHPO_4 . Maximum density of osseous tissue depends upon limitation of either the size or number of crystals or both, as bone mineral can only replace an equivalent volume of water; deposition of mineral cannot add to the total of the volume of the tissue being calcified (91). There is no adequate explanation for the limitation in size of the crystals. Poisoning of the surfaces of the crystals by extraneous ions has been suggested for the conditions *in vivo*; this does not explain the failure of crystals to attain greater size in aqueous solutions uncontaminated with such ions *in vitro*.

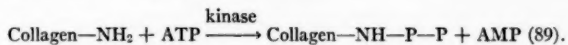
Enzymes and calcification.—The first enzyme to be implicated in the calcification mechanism was alkaline phosphatase, when Robison, in 1923, demonstrated it in bone [Bourne (5, Chap. 9)]. His explanation was that it liberates phosphate ions from organic combination, thus freeing them to combine with calcium. Robison himself abandoned this oversimplified version of the mechanism of calcification a few years later in favor of a more complicated system, and the original proposal has again been demonstrated to be untenable (11); it still dominates much of the literature. Alternate hypotheses for the role of phosphatase in bone have been presented [Bourne (5, Chap. 9)]; the one receiving the most support is that it participates in the production of a calcifiable protein matrix and that its function has to do with the property of calcifiability rather than with calcification per se (4). Histochemical studies of alkaline phosphatase as related to the biology of bone tissue have demonstrated the enzyme in osteoblasts and osteoclasts. Alkaline phosphatase is said to be not only the enzyme of osteogenesis, but also that of bone remodeling (92). Hypophosphatasia has been described in

infants and children and a considerable number of cases have been reported (93). It is associated with low alkaline phosphatase activity in the serum, bones, kidneys, and other tissues, and with defective bone formation.

A phosphorylase in calcifying cartilage catalyzes the breakdown of glycogen to glucose-1-phosphate. It has been believed to synthesize potential substrates for phosphatase in zones of calcification before blood sources become available, as well as for supplementing these blood sources thereafter. A study of the relations of glycogen, phosphorylase, and ground substance has led to the conclusion that the consumption of glycogen through the activity of phosphorylase may be associated with the production of bone matrix and rachitic osteoid and with the production or alteration of cartilage matrix prior to calcification (94).

The idea that calcification depends in some way upon the alkaline phosphatase of bone has been broadened to include the entire glycolytic cycle, beginning with phosphorylative glycogenolysis, in the mechanism of calcification. The early proposal that phosphorylation of glycogen, with the subsequent steps in glycolysis all involving phosphorylated intermediates, led eventually to a substrate for the action of phosphatase has been seen to run into a dead end (4). Such a substrate does not appear in the cycle, and incorporation of a phosphate ion into the cycle, with its subsequent release, does not increase the amount or concentration of this ion available for calcification. Not all of the steps in the glycolytic cycle have been verified for calcifying cartilage, but enough enzyme activities and intermediates have been identified to lead to the conclusion that the glycolytic systems of muscle and of calcifying cartilage are very similar if not identical (95). Proof that the cycle plays a specific part in calcification is still lacking (96).

There is current interest in the possible role of ATP, also concerned in the glycolytic cycle; it has many other functions as well and is now said to serve as a phosphate donor in calcification (84, 85, 89). It is pointed out that ATP is the only acid-soluble ester which accumulates as a result of the glycogenolysis in ossifying cartilage (97). The interest in ATP is strengthened by the demonstration of pyrophosphate as a constituent of normal bone. Since inorganic pyrophosphate is not an effective calcifying agent, it has been suggested that the role of ATP is that of transferring pyrophosphate to some component of the organic matrix with a transference mechanism; the working hypothesis is that a transphosphorylation reaction takes place in ossifying bone according to the scheme:



Linked with this proposal is the belief that the glycolytic cycle is important for the ossification process only for its ability to form pyruvic acid, by the oxidation of which the cartilage cell may synthesize ATP (98). Because of the role of insulin in the multienzyme system which controls the metabolism of ATP and the biochemical reactions dependent upon it, e.g.,

biosynthesis of chondroitin sulfate (99), attention is currently being directed to the influence of insulin upon calcification and ossification (100). The glycolytic cycle in the epiphyseal cartilage of the rat has been studied in relation to the rate of growth and to calcification (101).

TRACER METHODS

A kinetic approach to the interchange of ions, more especially of calcium and phosphate, between blood and bone, has been made possible by the use of radioisotopes. While use of P^{32} dates back to the papers of Hevesy, beginning in 1935, Ca^{45} was not available in quantities adequate to meet the needs in biology until 1948. Still more recently a beginning has been made in Sweden with Ca^{47} (102). This is not yet in general use, but its short half life and high-energy γ -radiation make it suitable for external counting and safe for use in man. Sr^{85} has much the same properties and has been used more extensively, but since the body discriminates between calcium and strontium (103 to 106) the latter is not an entirely reliable indicator of the behavior of calcium.

Ion transfer and ion exchange.—An important result of kinetic analysis of the movement of radiocalcium in the organism has been an appreciation of the rapidity of movements between blood and bone. Since radioactive ions taken up by bone either by accretion or by exchange are far outnumbered by the corresponding stable ions in the bone, the usual result is that a radioactive ion entering the bone mineral replaces a stable ion which is then released to the circulation. The net result gives the appearance of an irreversible uptake of the ion by bone (107), while in fact the apparent irreversibility concerns only the radioactive tracer. This appearance is further enhanced by the migration of such ions as Ca^{++} , Sr^{++} , and Ra^{++} into the interior of the crystals (11). It has been found that after approximately 52 days nearly all of the radiocalcium remaining in the body of a rat is located in the stable fraction of the bone mineral (108).

Skeletal dynamics.—Given an effective mechanism for nucleation or seeding and given adequate transport of calcium and phosphate to the skeleton, coupled with concentration of their ions in the fluids of the body above a critical level, calcification occurs. This statement defines the requirements for the local mechanism and the humoral factors in the mineralization of a calcifiable tissue. With the aid of radioisotopes there are now increasingly frequent attempts to define the kinetics of the movements of the ions concerned in this process.

Such studies began with the construction of curves illustrating the disappearance from the blood of an isotope, now commonly Ca^{45} , following its intravenous administration (109, 110, 111). These curves were then subjected to kinetic analysis and expressed as a composite of exponential decay curves. From such data there have been calculated the turnover rates of the calcium ions in the blood, with the surprising result that in young animals this rate may be as high as 100 per cent of the total amount in the blood per minute (112).

A wide field was opened for investigation by further extension of tracer techniques when, with the aid of computations based on blood disappearance curves and on observations of uptake of Ca^{45} by the bones of experimental animals, it became possible to calculate the rates of accretion, resorption, and exchange reactions in the skeleton (113). The methods were used to evaluate these processes under various physiologic and pathologic conditions and, in support of previous work, it was concluded that bone resorption requires the presence of vitamin D for its normal homeostatic function.

Similar calculations have been applied to the kinetics of the metabolism of Sr^{85} in man (114), using external counting methods for accumulation of data. By compartmental analysis, computations of the rates of movement of the isotope between the blood, intracellular fluid, extracellular fluid, and bone have been made. Similar studies are now in progress with Ca^{47} and are being extended to considerable numbers of patients with disorders of bone (102). Other studies have been reported on the time course of the concentration of intraperitoneally injected radiocalcium in the serum, skeleton, and other tissues of 10-day-old rats, with construction of a model of a multi-compartment system, in which the blood is the central or feeding compartment (107). Compartmental contents and transfer rates of phosphorus have been studied in the rat by the use of P^{32} as the tracer element (115).

REGULATORY PROCESSES AND BONE

Consideration of the regulatory processes affecting bone requires attention to the interplay of the physiologic factors in the growth, mineralization, turnover, and maintenance of the integrity of skeletal tissues. And since bone is so closely linked with the homeostatic control of the internal environment, more especially of its content of calcium and phosphate but including sodium and magnesium as well, it is not possible to consider the factors influencing the mineral of bone without attention to the bone-blood relationships.

Growth hormone.—Of the hormones known to influence growth, including androgens, estrogens, adrenocortical hormones, thyroxine, and insulin, only the growth hormone of the anterior lobe of the pituitary gland exhibits a specific effect upon the growth apparatus by means of which growth in length of the long bones continues up to closure of the epiphyses. Recent work on the chemistry and metabolic effects of pituitary growth hormone, from bovine and other sources, has been reviewed in detail (116, 117) [Asling and Evans (5, Chap. 21)]. The bovine preparations have given equivocal effects in man and have not been shown to induce growth in pituitary dwarfs. A more recent development has been the extraction of the hormone from primate sources (118 to 120), including human pituitary glands, and its clinical trial. In one instance (121), the hormone from human material, administered to a pituitary dwarf over a period of 11 months, led to a growth rate of 2.6 in. per year. An effect of insulin upon the growth of rats has been demonstrated, strengthening the possibility that decreased liberation of insulin may be one of the factors limiting growth in hypophysectomized animals (122).

The parathyroid-vitamin D complex.—The physiology of the parathyroids (123, 124) and of vitamin D (125) has been reviewed in recent years. For present purposes it is convenient to give special attention to the interrelations of the hormone and the vitamin, particularly with reference to their influence on the mineral of bone and upon the homeostatic control of the calcium ion concentration in the circulating fluids of the body.

There is now virtually unanimous agreement that the parathyroid hormone has a direct effect on bone, influencing its resorption and the release of its mineral constituents [McLean (5, Chap. 22)], (126). This does not exclude an effect upon the renal excretion of phosphates, exerted mainly on tubular reabsorption; such an effect is well established (127). In addition to the effect of vitamin D on absorption from the intestines, also well established (128), the concept of a local effect on bone, promoting deposition of the bone mineral, has given way to one of a calcium-mobilizing effect aiding in the homeostatic control of the calcium ion concentration in the blood plasma (129 to 131).

More recent developments have been those implicating the synthesis and metabolism of citric acid in the physiology of the parathyroids and of the action of vitamin D, leading to the proposal that citric acid is formed in the bone tissue under these two influences and that by the formation of soluble calcium-citrate complexes it plays an important part in the mobilization of calcium from the bones and in its transfer from bone to blood (11).

Homeostatic control of calcium ion concentration in the blood.—Introduction of tracer methods into the study of ion exchange between bone and blood (132) has led to new concepts concerning homeostatic regulation of blood calcium levels (123). In spite of a disappearance rate of calcium from the blood as high as 100 per cent per minute in young animals (112), the organism is able to maintain the concentration of calcium ions in the blood plasma at an approximately constant level. That the parathyroid glands monitor the calcium ion concentration in the plasma and that they participate in its regulation by means of a feedback mechanism which controls the release of calcium from bone to blood is well known (123). However, demonstration of the rapidity of the turnover of calcium in the blood has made it clear that the relatively slow-acting parathyroid control, while responsible for hour-to-hour or day-to-day adjustments, is not alone adequate for minute-to-minute interplay between blood and bone (20).

It has thus been necessary to postulate a dual mechanism for homeostatic control. One part, which acts rapidly, requires ion transfer or ion exchange between blood and bone and is independent of the function of the parathyroid glands, being able in the absence of these glands to maintain the plasma calcium level at approximately 7 mg. per 100 ml. (133). Whether this part of the mechanism acts passively, by physicochemical means, as has been assumed (123), or whether it requires cellular activity, under the control of the parathyroid-vitamin-D complex acting through an influence upon citrate metabolism (11), is not yet clear. In any case the deposition of

mineral in bone when there is an excess in the blood appears to require no regulatory mechanism, and the transfer of ions that takes place over periods of seconds or minutes between blood and bone now seems to be definitely localized in the accessible, labile, and reactive bone, mainly in new and incompletely mineralized osteons (79).

The second part of the dual mechanism, that which acts by feedback through the parathyroid glands and is responsible for maintenance of the normal serum calcium level, has access to the stable fraction of the bone mineral (134), by control of osteoclastic resorption, which occurs exclusively at surfaces of bone. Further clarification of the physiologic effects of the parathyroid hormone is in prospect, owing to current progress in its purification (135, 136).

Vitamin D.—After a period of relative inactivity, numerous contributions on the mode of action of vitamin D are now appearing, including studies at the cellular level (137). Within the period covered by this review, the concept of a local action of vitamin D on bone, participating in the mobilization of bone mineral, perhaps through an influence upon citrate metabolism, has been introduced (129, 130, 131). An oversimplified version of current views would be to say that vitamin D exerts an effect upon mobilization of calcium from bone in proportion to its intake and that the parathyroid hormone acts synergistically in this respect. Since parathyroid activity is influenced by the calcium ion level in the blood, the parathyroids are called upon to balance the interplay between dietary intake, urinary and fecal excretion, and the regulatory effect of vitamin D. The enlargement of the parathyroid glands frequently observed in animals on diets deficient in both calcium and vitamin D, and often attributed to the deficiency in calcium, is now said to result primarily from the deficiency in vitamin D, since hypertrophy of the parathyroids did not occur in rats fed diets very low in calcium but with an adequate vitamin-D supplement (138).

That excessive amounts of vitamin D in the diet may lead to hypercalcemia, and that this results from increased mobilization of bone mineral is well established (125). This occurrence has frequently been reported in the past, mainly in children. More recently there has been a series of reports of a newly recognized condition in infants, called idiopathic hypercalcemia of infancy (139, 140), attributed by some observers to hypersensitivity to vitamin D in the ordinary prophylactic dosage range; this hypersensitivity is said to lead to increased absorption of calcium from the gut (141). A further development, observed also in some patients, has been the demonstration of an antagonism between the effects of adrenocortical hormones and vitamin D; administration of cortisone has resulted in a lowering of the plasma calcium to normal levels (142). Hypocalcemic states in infancy and childhood, often requiring vitamin-D therapy, have been recently reviewed (143).

The current trend is to regard an increasing number of activated sterols, including dihydrotachysterol, as members of the vitamin D family. These

sterols differ quantitatively in their physiologic actions, and individuals differ in their response to them; it has been reported that when resistance to one form of vitamin D develops in the course of therapy, substitution of another form may lead to favorable results (144).

The citric acid of bone.—It is clear that the regulatory mechanisms which include the parathyroid hormone and vitamin D play a major part in the control of the metabolism of calcium and of citrate; the direct interrelations between calcium and citrate in bone and in the fluids of the body are less well understood. There is at present no adequate basis for deciding what may be the source or fate of circulating citrates, or to what extent the citrates in bone may play an active role in the metabolism of this tissue (145). The relationship of citrate to the physiology of bone has been recently reviewed [Dixon and Perkins (5, Chap. 11)]. One view is that some and perhaps the major part of the skeletal mineral citrate is present merely adventitiously, owing to the presence in body fluids of citrate derived from nonskeletal tissues and to their uptake by the bone mineral [Armstrong (6)]. That the bone mineral does take up citrate from the surrounding fluid has been demonstrated for calcification *in vitro* (146). On the other hand, a strong case has been made for the opposite view, i.e., that most of the citrate in the skeleton originates in the bones and that it is actively concerned in the transfer of calcium to the blood (11).

Hypoparathyroidism leads to a lowering of citrate concentration in bones and in blood, as well as to hypocalcemia; hyperparathyroidism or administration of parathyroid extract elevates both the citrate and calcium levels (147). The constancy of these findings suggests that there is a causal relationship between calcium and citrate levels. The evidence for this has been examined in detail (11).

Similarly, vitamin-D deficiency lowers the citrate concentration in bones and blood even though the serum calcium may be maintained at a normal level by parathyroid activity. Vitamin D, in sufficient dosages, raises the citrate level in the bones and in the fluids of the body while leading also to hypercalcemia (148). The mechanisms of parathyroid and of vitamin-D activity are not identical (11), and the two do not substitute for each other in every respect (147). Analyses for organic acids in bone, other than citric, have been reported (149).

Cations other than calcium.—In addition to calcium, which is the major cation in bone, the skeleton serves as a storage reservoir for many cations (150 to 152). Those of physiologic importance are chiefly sodium and magnesium. Of the sodium in bone, some 40 to 45 per cent is exchangeable and is readily available to the fluids of the body (153 to 157). The remainder is associated with the stable fraction of the bone mineral and is nonaccessible. Magnesium is presumably chemisorbed on the surfaces of the crystals of bone mineral and is exchangeable, at least in large part (11, 158). No homeostatic mechanism for regulation of the exchange of these ions between blood and bone is known. Potassium, although ubiquitous in the body, appears to have no special affinity for bone (156).

A considerable number of foreign cations are found as contaminants of the bone mineral when they reach the blood stream. Of these the most important are radium, strontium, and lead, all of which may substitute for calcium in the crystal structure of hydroxyapatite (11). Much recent attention has been directed to the harmful effects of the radioactivity of strontium and of radium, when incorporated within bone (103, 159, 160, 161) [Vaughan (5, Chap. 23)].

Rickets and osteomalacia.—Excellent reviews and monographs on rickets, osteomalacia, and related disorders have appeared recently (162 to 165). Attention is currently concentrated largely on disorders with skeletal manifestations of rickets or osteomalacia, but resistant to therapy with vitamin D. Various forms have been described and attributed to inadequate reabsorption of phosphate by renal tubules, leading to a persistent hypophosphatemia [Fanconi (6)]. Microradiographic studies of the bones in refractory rickets have led to the observation that the structural features differ from those in ordinary rickets, and the conclusion that the condition is a genetically determined entity. Treatment with massive doses of vitamin D is commonly employed, with some success. The improvement so obtained has been attributed to the hypercalcemic effects of the large doses of vitamin D; this treatment does not influence the morphology and does not cure the disease (166).

Vitamins A and C.—The effects of avitaminosis A, hypervitaminosis A (167), and ascorbic acid deficiency have been reviewed (168) [Barnicot and Datta (5, Chap. 17), Bourne (5, Chap. 18)].

Hormonal control of the integrity of the organic matrix of bone.—Since the physiologic turnover of bone affects both the mineral and organic constituents of bone, it follows that there must be continuous destruction and rebuilding of the organic matrix. It appears that collagen itself is slightly, if at all, subject to metabolic turnover (30, 31, 32); it requires replacement only when destroyed by resorption. From the point of view of the parathyroid hormone, destruction of the organic matrix must be regarded as incidental to the resorption that occurs in response to the need of the fluids of the body for calcium.

Once resorption has occurred, rebuilding follows; this is best seen in the Haversian remodeling of compact bone (169). There is an increasing body of evidence that this rebuilding is under hormonal control and indeed that there exists an antianabolic as well as an anabolic factor. The current view is that the sex hormones—estrogens and androgens—provide the anabolic factors and that the adrenocortical hormones—cortisone and hydrocortisone—are antianabolic in their effects (170). A search is in progress for substances with the anabolic effects of the androgenic steroids but with minimal androgenic activity (171). It is believed that there is an interplay between the effects of the sex hormones and of the adrenocortical hormones which, under normal circumstances, results in a balance between matrix destruction and matrix rebuilding. If, however, the antianabolic effects of cortisone predominate, the result is failure of replacement of matrix, leading to a deficiency in organic material. This argument forms the basis of the widely

accepted theory of the pathogenesis of osteoporosis, and of the corresponding therapy by administration of the anabolic hormones—estrogens and androgens (172).

A weakness of this theory lies in the failure of anyone, and there have been many attempts, to demonstrate reconstruction of organic matrix, with its subsequent mineralization, by administration of estrogens and androgens or both. The explanation commonly advanced for this failure is the relative insensitivity of x-rays to changes in the density of bone. Against this may be weighed the fact that remineralization of the skeleton, with return to an approximation of normal density, has been demonstrated by x-rays following bilateral adrenalectomy in severe Cushing's disease, without administration of sex hormones (173).

A further weakness in this theory is that the use of estrogens and androgens or both in the treatment of osteoporosis had its origins in the observation that estrogens lead to formation of intramedullary bone in pigeons and in the later observation that a similar phenomenon can be produced in mice by administration of the female sex hormone (4). Albright & Reifenstein (174) state: "Our own studies in the human . . . are in agreement with the findings in pigeons." This statement rests on their demonstration that a positive nitrogen and calcium balance can be induced in man by administration of anabolic hormones; new bone formation induced by these hormones has never been satisfactorily demonstrated in any mammal except the mouse (175). It is perhaps significant that only in the mouse has osteoporosis been reported following ovariectomy (176). The medullary bone of laying birds (177), and the mechanism of the concurrent hypercalcemia (178) have been further studied. The influence of estrogens on the metabolism of sulfate-S³⁵ and of Ca⁴⁵ in the metaphyses of immature rats has been investigated (179).

There seems to be no doubt that estrogens and androgens or both produce a beneficial effect in many patients with osteoporosis, at least symptomatically, although the mechanism of this is not clear. It also appears that the detrimental effects of cortisone administration on the skeletons of some patients may be alleviated by simultaneous administration of estrogens and androgens or both (172). There is thus sufficient reason for accepting the idea that the amount and integrity of the organic matrix are under the control of an hormonal balance, although the simplest expression of this—cortisone vs. estrogens and androgens or both—does not seem to afford a completely satisfactory explanation of the observable phenomena.

Osteoporosis.—Some features of osteoporosis, at least in certain patients, can be explained on the basis of an imbalance between the effects of adrenocortical hormones and those of estrogens and androgens or both (180). This is clearly the case in Cushing's syndrome, in which osteoporosis results from overproduction of adrenocortical hormones (181) and has been treated successfully by removing the cause (173). The next step in the argument, i.e., that senile or postmenopausal osteoporosis is a *forme fruste* of Cushing's syndrome in that a diminution in output of sex hormones leads to a relative

hypercortisonism, while widely accepted, is by no means established. Moreover, the osteoporosis or atrophy of disuse, resulting from immobilization, by virtue of the rapid loss of bone substance with a flooding of the system with the calcium released from the bone mineral is even less readily explained on the basis of an antianabolic effect of cortisone (182). The difficulties of interpretation can be met, in part at least, by attributing a catabolic or destructive effect to cortisone, in addition to its role in antagonizing the anabolic effects of the sex hormones. The same may be said with reference to the osteoporosis following prolonged administration of cortisone and ACTH or both (183).

If failure to rebuild the organic matrix of bone is accepted as an essential feature of the pathogenesis of osteoporosis, factors others other than control by hormonal balance may be looked for. An obvious possibility is a deficiency in protein intake such as may occur in starvation or in malnutrition. This possibility has been supported by demonstration of a positive nitrogen balance under treatment with the anabolic hormones (174), and by favorable results attending feeding or parenteral administration of increased amounts of protein (184).

Very early studies of osteoporosis, with the demonstration of a reduction in total bone mass and of diminution in the density of the bones to x-ray, led to a belief that a deficient intake of calcium, especially when continued over a period of years, could be the determining factor in the occurrence of osteoporosis. But such bone as remains in the skeleton, even in advanced osteoporosis, has a mineral content within the normal range, and failure to calcify new matrix is rickets or osteomalacia rather than osteoporosis. The facts that such matrix as is formed calcifies readily, and that deprivation of calcium and vitamin D or both leads to rickets or osteomalacia, form the pillars of the too-little-matrix concept (174) of osteoporosis and have led to the attempt to bring all of the diverse forms of this condition within the scope of one pathologic picture and one mechanism for its pathogenesis. Calcium deficiency is now rarely mentioned as a possible cause of osteoporosis.

LITERATURE CITED

1. Baker, B. L., and Abrams, G. D., *Ann. Rev. Physiol.*, **17**, 61-78 (1955)
2. Dorfman, A., and Mathews, M. B., *Ann. Rev. Physiol.*, **18**, 69-88 (1956)
3. Dallemagne, M. J., *Ann. Rev. Physiol.*, **12**, 101-18 (1950)
4. McLean, F. C., and Urist, M. R., *Bone: An Introduction to the Physiology of Skeletal Tissue* (University of Chicago Press, Chicago, Ill., 182 pp., 1955)
5. Bourne, G. H., Ed., *The Biochemistry and Physiology of Bone* (Academic Press, Inc., New York, N. Y., 875 pp., 1956)
6. Wolstenholme, G. E. W., and O'Connor, C. M., Eds., *Ciba Foundation Symposium; Bone structure and Metabolism* (Little, Brown and Co., Boston, Mass., 299 pp., 1956)
7. U. S. Armed Forces Med. Library Reference Div., *The Structure, Composition and Growth of Bone 1930-1953*, A Bibliography compiled by Spencer, M. C., and Uhler, K. (Supt. Doc., Washington, D.C., 190 pp., 1955)

8. "Colloq. Ges. Physiol. Chem.," am. 12./14. April, 1956, Mosbach/Baden, *Chemie und Stoffwechsel von Binde- und Knochengewebe* (Springer-Verlag OHG, Berlin-Göttingen-Heidelberg, Germany, 142 pp., 1956)
9. Miner, R. W., Ed., *Ann. N. Y. Acad. Sci.*, **60**, 541-806 (1955)
10. Morse, K. T., and Furness, F. N., Eds., *Ann. N. Y. Acad. Sci.*, **64**, 279-462 (1956)
11. Neuman, W. F., and Neuman, M. W., *The Chemical Dynamics of Bone Mineral* (University of Chicago Press, Chicago, Ill., 209 pp., 1958)
12. Lacroix, P., *The Organization of Bones* (Transl. from amended French ed. by Gilder, S. The Blakiston Co., Philadelphia, Pa., 235 pp., 1951)
13. Irving, J. T., *Calcium Metabolism* (Methuen & Co., Ltd., London, Engl., and John Wiley & Sons, Inc., New York, N. Y., 177 pp., 1957)
14. Weinmann, J. P., and Sicher, H., *Bone and Bones, Fundamentals of Bone Biology*, 2nd ed. (C. V. Mosby Co., St. Louis, Mo., 508 pp., 1955)
15. *Conf. on Metabolic Interrelations, Trans. 1st, 2nd, 3rd, 4th, 5th Conf.* (Josiah Macy, Jr. Foundation, New York, N. Y., 1949, 1950, 1951, 1952, 1953)
16. Tunbridge, R. E., Ed., *Connective Tissue Symposium* (Charles C Thomas, Publisher, Springfield, Ill., 371 pp., 1957)
17. Engström, A., and Finean, J. B., *Biological Ultrastructure* (Academic Press, Inc., New York, N. Y., 326 pp., 1958)
18. Carlström, D. G., *J. Bone and Joint Surg.*, **39A**, 622-24 (1957)
19. Brown, R., and Danielli, J. F., Eds., *Symposia of the Soc. Exptl. Biol.*, No. IX, *Fibrous Proteins and Their Biological Significance* (Academic Press, Inc., New York, N. Y., 370 pp., 1955)
20. McLean, F. C., *Science*, **127**, 451-56 (1958)
21. Amprino, R., *Z. Zellforsch. u. mikroskop. Anat.*, **37**, 144-83 (1952)
22. Amprino, R., *Z. Zellforsch. u. mikroskop. Anat.*, **37**, 240-73 (1952)
23. Engström, A., Lagergren, C., and Lundberg, B., *Exptl. Cell Research*, **12**, 592-98 (1957)
24. Bondareff, W., *Gerontologia*, **1**, 222-33 (1957)
25. Asadi, A. M., Dougherty, T. F., and Cochran, G. W., *Nature*, **178**, 1061-62 (1956)
26. Schmitt, F. O., *J. Cellular Comp. Physiol.*, **49**, 85-104 (1957)
27. Martin, A. V. W., *Biochim. et Biophys. Acta*, **10**, 42-48 (1953)
28. Gross, J., *J. Biophys. Biochem. Cytol.*, **2**, Suppl., 261-74 (1956)
29. Robinson, R. A., and Watson, M. L., *Anat. Record.*, **114**, 383-410 (1952)
30. Neuberger, A., and Slack, H. G. B., *Biochem. J.*, **53**, 47-52 (1953)
31. Thompson, R. C., and Ballou, J. E., *J. Biol. Chem.*, **223**, 795-809 (1956)
32. Robertson, W. van B., *J. Biol. Chem.*, **197**, 495-501 (1952)
33. Gersh, I., *Conference on Connective Tissues, Trans. 2nd Conf.*, 11-44 (Josiah Macy, Jr. Foundation, New York, N. Y., 1951)
34. Glegg, R. E., and Eidinger, D., *Arch. Biochem. Biophys.*, **55**,¹ 19-24 (1955)
35. McConnell, D., *Biochim. et Biophys. Acta*, **17**, 450-51 (1955)
36. Caglioti, V., Ascenzi, A., and Santoro, A., *Biochim. et Biophys. Acta*, **21**, 425-32 (1956)
37. Ascenzi, A., *Sci. med. ital.*, **3**, 670-97 (1955)
38. Sobel, A. E., and Hanok, A., *J. Dental Research*, **37**, 631-37 (1958)
39. Neuman, W. F., and Neuman, M. W., *Am. J. Med.*, **22**, 123-31 (1957)
40. Dallemagne, M. J., and Fabry, C., *Acta chir. belg.*, Suppl. 1, 75-114 (1956)
41. Speckman, T. W., and Norris, W. P., *Science*, **126**, 753 (1957)

42. Fernández-Morán, H., and Engström, A., *Biochim. et Biophys. Acta*, **23**, 260-64 (1957)
43. Carlström, D., and Finean, J. B., *Biochim. et Biophys. Acta*, **13**, 183-91 (1954)
44. Scott, B. L., and Pease, D. C., *Anat. Record*, **126**, 465-94 (1956)
45. Bauer, G. C. H., Carlsson, A., and Lindquist, B., *Acta Med. Scand.*, **158**, 143-50 (1957)
46. Jackson, S. F., *Proc. Roy. Soc. (London), Ser. B*, **146**, 270-80 (1957)
47. Sheldon, H., and Robinson, R. A., *J. Biophys. Biochem. Cytol.*, **3**, 1011-16 (1957)
48. Sobel, A. E., and Burger, M., *Proc. Soc. Exptl. Biol. Med.*, **87**, 7-13 (1954)
49. Meyer, K., Davidson, E., Linker, A., and Hoffman, P., *Biochim. et Biophys. Acta*, **21**, 506-18 (1956)
50. Wassermann, F., *Ergeb. Anat. u. Entwicklungsgeschichte*, **35**, 240-333 (1956)
51. Heller, M., McLean, F. C., and Bloom, W., *Am. J. Anat.*, **87**, 315-48 (1950)
52. Heller-Steinberg, M., *Am. J. Anat.*, **89**, 347-79 (1951)
53. Lipp, W., *Acta Anat.*, **22**, 151-201 (1954)
54. McLean, F. C., *J. Periodontol.*, **25**, 176-82 (1954)
55. Schatz, A., Karlson, K. E., Martin, J. J., and Schatz, V., *Ann. Dentistry*, **16**, 37-49 (1957)
56. Bhaskar, S. N., Mohammed, C. I., and Weinmann, J. P., *J. Bone and Joint Surg.*, **38A**, 1335-45 (1956)
57. Kroon, D. B., *Acta Anat.*, **21**, 1-18 (1954)
58. Cameron, D. A., and Robinson, R. A., *J. Bone and Joint Surg.*, **40A**, 414-18 (1958)
59. Arnold, J. S., and Jee, W. S. S., *Am. J. Anat.*, **101**, 367-418 (1957)
60. Park, E. A., *Arch. Disease Childhood*, **29**, 269-81 (1954)
61. Knese, K.-H., *Z. Zellforsch. u. mikroskop. Anat.*, **44**, 585-643 (1956)
62. Pritchard, J. J., *J. Anat.*, **86**, 259-77 (1952)
63. Cohen, J., and Harris, W. H., *J. Bone and Joint Surg.*, **40A**, 419-34 (1958)
64. De Bruyn, P. P. H., and Kabisch, W. T., *Am. J. Anat.*, **96**, 375-417 (1955)
65. Williams, R. G., *Anat. Record*, **129**, 187-210 (1957)
66. Chase, S. W., and Herndon, C. H., *J. Bone and Joint Surg.*, **37A**, 809-41 (1955)
67. Danis, A., *Acta orthop. belg.*, **23**, 408-13 (1957)
68. Ray, R. D., Degge, J., Gloyd, P., and Mooney, G., *J. Bone and Joint Surg.*, **34A**, 638-47 (1952)
69. Urist, M. R., and McLean, F. C., *J. Bone and Joint Surg.*, **34A**, 443-70 (1952)
70. Ray, R. D., and Holloway, J. A., *J. Bone and Joint Surg.*, **39A**, 1119-28 (1957)
71. Hurley, L. A., and Losee, F. D., *Military Med.*, **121**, 101-4 (1957)
72. Holmstrand, K., *Acta Orthop. Scand.*, Suppl. 26, 66 pp. (1957)
73. Moss, M. L., *Science*, **127**, 755-56 (1958)
74. Tucker, E. J., *Surg., Gynecol. Obstet.*, **96**, 739-49 (1953)
75. Duthie, R. B., *Brit. J. Plastic Surg.*, **11**, 1-30 (1958)
76. Lacroix, P., *Proc. Radioisotope Conf., 2nd Conf.*, Oxford, 1954, **1**, 134-37 (Butterworths Scientific Publications, London, Engl., 1954)
77. Vincent, J., *Rev. belge. pathol. et méd. exptl.*, **26**, 161-68 (1957)
78. Lacroix, P., *Bull. acad. roy. méd. Belg.*, **18**, 489-96 (1953)
79. Marshall, J. H., Rowland, J. E., and Jowsey, J., *Radiation Research* (In press, 1958)
80. Amprino, R., *Ann. soc. sci. méd. nat. Bruxelles*, **4**, 209-25 (1951)
81. Levinskas, G. J., and Neuman, W. F., *J. Phys. Chem.*, **89**, 164-68 (1955)

82. Robinson, R. A., and Cameron, D. A., *J. Biophys. Biochem. Cytol.*, **2**, Suppl., 253-60 (1956)
83. DiStefano, V., Neuman, W. F., and Rouser, G., *Arch. Biochem. Biophys.*, **47**, 218-20 (1953)
84. Picard, J., *Relations entre la minéralisation du cartilage ossifiable et son métabolisme chez l'embryon de mouton et le rat* (Thesis, Faculté de Médecine, Hôpital des Enfants Malades, Paris, France, 170 pp., 1955)
85. Cartier, P., and Picard, J., *Bull. soc. chim. biol.*, **37**, 1159-68 (1955)
86. Glimcher, M. J., Hodge, A. J., and Schmitt, F. O., *Proc. Natl. Acad. Sci. U. S.*, **43**, 860-67 (1957)
87. Gersh, I., *Harvey Lectures*, Ser. XIV, 211-41 (1950)
88. Miller, Z. B., Waldman, J., and McLean, F. C., *J. Exptl. Med.*, **95**, 497-508 (1952)
89. Zambotti, V., *Sci. med. ital.*, **5**, 614-43 (1957)
90. Perloff, A., and Posner, A. S., *Science*, **124**, 583-84 (1956)
91. Robinson, R. A., and Elliott, S. R., *J. Bone and Joint Surg.*, **39A**, 167-88 (1957)
92. Majno, G., and Rouiller, C., *Arch. pathol. Anat. u. Physiol., Virchow's*, **321**, 1-61 (1951)
93. Dickson, W., and Horrocks, R. H., *J. Bone and Joint Surg.*, **40B**, 64-74 (1958)
94. Cobb, J. D., *Arch. Pathol.*, **55**, 496-502 (1953)
95. Albaum, H. G., Hirshfeld, A., and Sobel, A. E., *Proc. Soc. Exptl. Biol. Med.*, **79**, 682-86 (1952)
96. Sobel, A. E., Burger, M., Deane, B. C., Albaum, H. G., and Cost, K., *Proc. Soc. Exptl. Biol. Med.*, **96**, 32-39 (1957)
97. Whitehead, R. G., and Weidman, S. M., *Nature*, **180**, 1196-97 (1957)
98. Barbieri, E., *Clin. Orthop.*, **9**, 595-602 (1957)
99. Barbieri, E., *Experientia*, **13**, 370-71 (1957)
100. Siliprandi, N., De Bastiani, G., Petrelli, F., and Fischetti, B., *Nature*, **180**, 1357-58 (1957)
101. Eeg-Larsen, N., *Acta Physiol. Scand.*, **38**, Suppl. 128, 77 pp. (1956)
102. Bauer, G. C. H., *Proc. UNESCO Intern. Conf. on Radioisotopes in Sci. Research*, Paris, Sept. 11-20, 1957 (Exterman, R. C., Ed., Pergamon Press, London, Engl., **4**, 232-46 (1958))
103. Engström, A., Björnerstedt, R., Clemenson, C. -J., and Nelson, A., *Bone and Radiostrontium* (Almqvist & Wiksell, Stockholm, Sweden; John Wiley & Sons, Inc., New York, N. Y., 139 pp., 1958)
104. Comar, C. L., Wasserman, R. H., Ullberg, S., and Andrews, G. A., *Proc. Soc. Exptl. Biol. Med.*, **95**, 386-91 (1957)
105. Spencer, H., Laszlo, D., and Brothers, M., *J. Clin. Invest.*, **36**, 680-88 (1957)
106. Alexander, G. V., Nusbaum, R. E., and MacDonald, N. S., *J. Biol. Chem.*, **218**, 911-19 (1956)
107. Bronner, F., *J. Gen. Physiol.*, **41**, 767-82 (1958)
108. Singer, L., and Armstrong, W. D., *Proc. Soc. Exptl. Biol. Med.*, **76**, 229-33 (1951)
109. Thomas, R. O., Litovitz, T. A., Rubin, M. I., and Geschickter, C. F., *Am. J. Physiol.*, **169**, 568-75 (1952)
110. Armstrong, W. D., Johnson, J. A., Singer, L., Lienke, R. I., and Premer, M. L., *Am. J. Physiol.*, **171**, 641-51 (1952)
111. Bronner, F., Harris, R. S., Maletskos, C. J., and Benda, C. E., *J. Clin. Invest.*, **35**, 78-88 (1956)
112. Hansard, S. L., Comar, C. L., and Davis, G. K., *Am. J. Physiol.*, **177**, 383-89 (1954)

113. Bauer, G. C. H., Carlsson, A., and Lindquist, B., *Kungl. Fysiograf. Sällskapets I Lund Förhandl.*, **25**, 1-16 (1955)
114. Bauer, G. C. H., and Ray, R. D., *J. Bone and Joint Surg.*, **40A**, 171-86 (1958)
115. Lax, L. C., Sidlofsky, S., and Wrenshall, G. A., *J. Physiol. (London)*, **132**, 1-19 (1956)
116. Smith, R. W., Jr., Gaebler, O. H., and Long, C. N. H., Eds., *International Symposium on the Hypophyseal Growth Hormone, Nature and Actions* (McGraw-Hill Book Co., Inc., New York, N. Y., 576 pp., 1955)
117. Russell, J. A., and Wilhelmi, A. E., *Ann. Rev. Physiol.*, **20**, 43-76 (1958)
118. Beck, J. C., McGarry, E. E., Dyrenfurth, I., and Venning, E. H., *Science*, **125**, 884-85 (1957)
119. Raben, M. S., *Science*, **125**, 883-84 (1957)
120. Knobil, E., Morse, A., Wolf, R. C., and Greep, R. O., *Endocrinology*, **62**, 348-54 (1958)
121. Raben, M. S., and Hollenberg, C. H., *J. Clin. Invest.*, **37**, 922-23 (1958)
122. Salter, J., and Best, C. H., *Brit. Med. J.*, **II**, 353-56 (1953)
123. McLean, F. C., *Clin. Orthop.*, **9**, 46-60 (1957)
124. Bartter, F. C., *Ann. Rev. Physiol.*, **16**, 429-44 (1954)
125. Nicolaysen, R., and Eeg-Larsen, N., *Vitamins and Hormones*, **11**, 28-60 (1953)
126. Howard, J. E., *J. Clin. Endocrinol.*, **17**, 1105-23 (1957)
127. Chambers, E. L., Jr., Gordan, G. S., Goldman, L., and Reifenshtein, E. C., Jr., *J. Clin. Endocrinol.*, **16**, 1507-21 (1956)
128. Nicolaysen, R., Eeg-Larsen, N., and Malm, O. J., *Physiol. Revs.*, **33**, 424-44 (1953)
129. Carlsson, A., *Acta Physiol. Scand.*, **26**, 212-20 (1952)
130. Lindquist, B., *Acta Paediat.*, **41**, Suppl. 86, 82 pp. (1952)
131. Carlsson, A., and Hollunger, G., *Acta Physiol. Scand.*, **31**, 317-33 (1954)
132. Comar, C. L., and Wasserman, R. H., *Progr. in Nuclear Energy, Series VI, Biol. Sci.*, **1**, 153-96 (1956)
133. Copp, D. H., *Am. J. Med.*, **22**, 275-85 (1957)
134. Woods, K. R., and Armstrong, W. D., *Proc. Soc. Exptl. Biol. Med.*, **91**, 255-58 (1956)
135. Rasmussen, H., *J. Biol. Chem.*, **229**, 781-87 (1957)
136. Rasmussen, H., and Westall, R. G., *Biochem. J.*, **67**, 658-63 (1957)
137. De Luca, H. F., Gran, F. C., Steenbock, H., and Reiser, S., *J. Biol. Chem.*, **228**, 469-74 (1957)
138. Crawford, J. D., Gribetz, D., Diner, W. C., Hurst, P., and Castleman, B., *Endocrinology*, **61**, 59-71 (1957)
139. Fanconi, G., and Girardet, P., *Helv. Paediat. Acta*, **7**, 314-34 (1952)
140. Schlesinger, B., Butler, N., and Black, J., *Helv. Paediat. Acta*, **7**, 335-49 (1952)
141. Bonham Carter, R. E., Dent, C. E., Fowler, D. I., and Harper, C. M., *Arch. Disease Childhood*, **30**, 399-404 (1955)
142. Anderson, J., Dent, C. E., Harper, C., and Philpot, G. R., *Lancet*, **II**, 720-24 (1954)
143. Gribetz, D., *J. Diseases Children*, **94**, 301-12 (1957)
144. Dent, C. E., Harper, C. M., Morgans, M. E., Philpot, G. R., and Trotter, W. R., *Lancet*, **II**, 687-90 (1955)
145. Elliott, J. R., and Freeman, S., *Endocrinology*, **59**, 181-89 (1956)
146. Sobel, A. E., Goldenberg, H., and Schmerzler, E., *J. Dental Research*, **33**, 497-503 (1954)
147. Elliott, J. R., and Freeman, S., *Endocrinology*, **59**, 196-200 (1956)

148. Harrison, H. C., Harrison, H. E., and Park, E. A., *Am. J. Physiol.*, **192**, 432-36 (1958)
149. Lees, H., and Kuyper, A. C., *J. Biol. Chem.*, **225**, 641-49 (1957)
150. Duckworth, J., and Hill, R., *Nutrition Abstr. & Revs.*, **23**, 1-17 (1953)
151. Owen, E. C., *Brit. J. Nutrition*, **6**, 415-23 (1952)
152. Bergstrom, W. H., *Metabolism, Clin. and Exptl.*, **5**, 433-37 (1956)
153. Bauer, G. C. H., *Acta Physiol. Scand.*, **31**, 334-50 (1954)
154. Edelman, I. S., James, A. H., Baden, H., and Moore, F. D., *J. Clin. Invest.*, **33**, 122-31 (1954)
155. Nichols, G., Jr., and Nichols, N., *Metabolism, Clin. and Exptl.*, **5**, 438-46 (1956)
156. Stoll, W. R., and Neuman, W. F., *J. Am. Chem. Soc.*, **78**, 1585-88 (1956)
157. Forbes, G. B., Mizner, G. L., and Lewis, A., *Am. J. Physiol.*, **190**, 152-56 (1957)
158. Fitzgerald, M. G., and Fourman, P., *Clin. Sci.*, **15**, 635-47 (1956)
159. Aub, J. C., Evans, R. D., Hempelmann, L. H., and Martland, H. S., *Medicine*, **31**, 221-329 (1952)
160. Looney, W. B., *Science*, **127**, 630-33 (1958)
161. Owen, M., Sissons, H. A., and Vaughan, J., *Brit. J. Cancer*, **11**, 229-48 (1957)
162. Snapper, I., and Nathan, D. J., *Am. J. Med.*, **22**, 939-48 (1957)
163. Snapper, I., *Bone Diseases in Medical Practice* (Grune & Stratton, Inc., New York, N. Y., 229 pp., 1957)
164. Follis, R. H., Jr., *Am. J. Med.*, **22**, 469-84 (1957)
165. Uehlinger, E., *Verhandl. deut. Ges. inn. Med.*, **62**, 368-403 (1956)
166. Engfeldt, B., Zetterström, R., and Winberg, J., *J. Bone and Joint Surg.*, **38A**, 1323-34 (1956)
167. Breslau, R. C., *Arch. Pediat.*, **74**, 139-52; 178-97 (1957)
168. Irving, J. T., *Vitamins and Hormones*, **15**, 291-323 (1957)
169. Vincent, J., *Arch. biol. (Liège)*, **68**, 561-79 (1957)
170. Reifenshtein, E. C., Jr., *Southern Med. J.*, **49**, 933-60 (1956)
171. Gordan, G. S., *Arch. Internal Med.*, **100**, 744-49 (1957)
172. Reifenshtein, E. C., Jr., *Clin. Orthop.*, **9**, 75-84 (1957)
173. Skeels, R. F., *J. Clin. Endocrinol.*, **18**, 61-64 (1958)
174. Albright, F., and Reifenshtein, E. C., Jr., *The Parathyroid Glands and Metabolic Bone Disease* (Williams and Wilkins Co., Baltimore, Md., 393 pp., 1948)
175. Budy, A. M., *Ann. N. Y. Acad. Sci.*, **64**, 428-31 (1956)
176. Piroshaw, N. A., and Glickman, I., *Oral Surg., Oral Med., Oral Pathol.*, **10**, 133-47 (1957)
177. Bloom, M. A., Domm, L. V., Nalbandov, A. V., and Bloom, W., *Am. J. Anat.*, **102**, 411-54 (1958)
178. Urist, M. R., Schjeide, O. A., and McLean, F. C., *Endocrinology*, **63**, 570-85 (1958)
179. Dziewiatkowski, D., Bronner, F., Di Ferrante, N., and Archibald, R. M., *J. Biophys. Biochem. Cytol.*, **3**, 151-60 (1957)
180. Bartter, F. C., *Am. J. Med.*, **22**, 797-806 (1957)
181. Sissons, H. A., *J. Bone and Joint Surg.*, **38B**, 418-33 (1956)
182. Whedon, G. D., and Shorr, E., *J. Clin. Invest.*, **36**, 966-81 (1957)
183. Good, R. A., Vernier, R. L., and Smith, R. T., *Pediatrics*, **19**, 95-118; 272-84 (1957)
184. Gardner, A. L., *Am. Practitioner and Digest. Treatment*, **9**, 767-79 (1958)

HEAT AND COLD¹

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Temperature is one of the most important factors that determine life on earth. Among numerous means developed by living organisms to exist under various thermal conditions, "thermodynamic freedom" (61) of homeotherms is without doubt the most ingenious achievement. According to the reviewer's interests, this review is devoted mainly to temperature regulation, its limits, and its slow changes. Despite a wealth of valuable experimental results, the fundamental processes of thermoregulation are far from understood, especially the nature of central thermal sensitivity and the integrating mechanisms. As to the basic problems of acclimatization, physiology has hardly left the era of mere description.

The practical importance of some aspects of thermophysiology, such as hypothermia, has led to an enormous increase of literature during the period covered by this review. In view of space limitation, an arbitrary selection of papers has been necessary. General problems of heat and cold in man and animals have been reviewed in recent years by Burton & Edholm (61), Burton (60), Precht, Christophersen & Hensel (254), Lee (208, 209), and Levitt (213); reviews on special topics will be mentioned in the respective sections.

HEAT EXCHANGES

Methods.—New climatic chambers have been described (130, 241). The type constructed by Müller & Wenzel (241) allows differences of 100°C. between air and radiant temperature without convection on the radiating surfaces. Brück & Hensel (55) designed a climatized respiration chamber for studying temperature regulation in newborn infants. Benzinger *et al.* (37) have further improved their gradient calorimeter. Basic work on heat transfer of living skin is being continued. Model experiments revealed considerable errors of surface temperature measurements by thermocouples as compared with radiometric methods (300); however, whether such comparisons may be properly applied to living human skin remains to be clarified, as the possibility of radiation sources below the skin surface cannot be excluded (247, 248). In order to calculate "thermal inertia" (product of heat conductivity and heat capacity) of living skin, Hendler *et al.* (153) recorded skin temperature radiometrically during infrared irradiation, whereas Vendrik & Vos (316) used skin temperature changes on contact with glass plates of known thermal properties. Values of 8.6 to 14×10^{-4} cal.² cm.⁻⁴ sec.⁻¹ °C.⁻² have been obtained (79, 147, 153, 316). A description of a microthermoneedle is given by Vere (317).

¹ The survey of literature pertaining to this review was concluded May 31, 1958.

Heat transfer.—The insulating effect of vasoconstriction in the human head and face is relatively small (108, 312). Froese & Burton (108) point out the importance of head protection in the cold, since 50 per cent of total heat loss may be given off from the head. Thermal comfort obtained from heated ceilings was studied by Wenzel & Müller (323). The irradiated body surface being relatively small, a raise in radiation temperature by 10°C . was necessary to compensate the physiological effect of an air temperature drop of 1°C . The human respiratory tract is demonstrated to have a considerable importance as a heat exchanger preventing heat losses to cold surroundings (319). Countercurrent heat exchange systems between arteries and veins allow nutritive blood flow to the extremities without transportation of heat to the surroundings. In whales and seals, countercurrent systems are found in the tail fluke and in the flippers (24, 175, 186). The most elaborate heat exchangers are the vascular bundles in the extremities of sloths, in which Scholander & Krog (282) have measured temperature gradients along the flow of $1^{\circ}\text{C}/\text{cm.}$, which is 30 times steeper than in the human arm.

Miller & Blyth (238) find no difference in heat transport to the body surface between fat and lean subjects during heat exposure. They conclude that the insulating effect of fat is negligible under these circumstances because practically all heat is transferred by convection. The diminished heat tolerance of fat people might be caused by factors other than fat insulation. In the seal, however, fat is a serious hindrance to heat loss during activity on shore (24). The high thermal insulation of fur from arctic animals has been demonstrated once more, but even the poorest fur from an opossum has proved superior to any pile fabric made to simulate fur. Replacing the air by freon led to a fourfold increase of thermal insulation (138). In U. S. Air Force protective clothing, humidity and hydrostatic compression in water reduce the insulation by 60 to 80 per cent (137).

The puzzling story of dry and damp cold has been studied by Burton *et al.* (62). When skin temperatures and heat losses were the same, dry cold caused a higher metabolic rate and a more pronounced cold sensation than did damp cold. This effect is discussed in connection with Bazett's gradient theory of thermoreceptor excitation; this concept is disproved, however, by recent investigations on cold receptor discharge (56, 161, 242). A series of measurements was made on spectral reflectance and distribution of radiant energy in the skin of man (8, 79, 146, 179, 180) and laboratory animals (8, 80, 147). According to Hardy *et al.* (146) and former investigators (80, 179), there is no influence of different pigmentation at wavelengths above $1.2\ \mu$. In this region the spectral absorption is primarily that of water. The current belief that an arctic animal may derive some advantages from being white, because of less radiative heat loss, has been definitively disproved (139).

Internal body temperatures.—Further evidence is adduced that practically no heat exchange takes place at the alveolar level. Bligh (43) measured the temperature difference between pulmonary artery and bicarotid trunk in the calf during thermal polypnoea. Despite considerable variations in air tem-

perature, the temperature difference never exceeded the measuring error of 0.05°C . The surprising finding of Good & Sellers (127) that the temperature in the left atrium of dogs in severe cold is even 0.01 to 0.15°C . higher than in the pulmonary artery might be explained partially by thermodynamics of gas exchanges.

In contrast to the current view that the highest temperature in the resting body is that of the liver (131), Graf & Graf (129) found the liver temperature in healthy resting subjects to be $0.18 \pm 0.03^{\circ}\text{C}$. below rectal temperature. This is probably due to a cooling effect of blood passing the liver; increased liver flow caused a temperature drop, the rectum-liver gradient being increased, whereas reduced liver flow had the opposite effect. In one single case, an increase of liver temperature above rectal temperature was seen after pyrogen injection.

HEAT PRODUCTION

In seals as bare-skinned animals, Irving & Hart (175) found critical temperatures in water from 20 to 0°C ., varying with body size and fat insulation. The skin temperature of only 1°C . raises problems concerning temperature reception, because in homeotherms no cold impulses are detected below 10°C . Are they completely abolished in the seal? What then causes the metabolic increase?

Chemical thermogenesis.—Chemical thermogenesis (heat production without visible or invisible muscular activity) contributes to a considerable degree to total heat production of rats in the cold. Donhoff *et al.* (84, 85) found a periodic increase in oxygen consumption with a coincident drop in muscle temperature and a rise in abdominal temperature. Davis & Mayer (77) estimated the chemical thermogenesis in the curarized rat to be about 40 per cent of the metabolic increase in the cold. This partition could be abolished by "internal" microwave heating in the curarized as well as in the normal animal in the cold, but no reduction of the remaining 60 per cent of physical thermogenesis was achieved by microwave heating. The adrenergic system plays an important role in stimulating chemical heat production (169, 170).

Shivering.—It has been suggested that shivering can be elicited from cutaneous receptors alone. This conclusion is drawn from experiments with man and animals (127, 128, 168, 297) showing a rapid onset of shivering in the cold without any drop, or even with a slight increase, in rectal or heart temperature. As hypothalamic temperature has not been recorded directly, these experiments are not conclusive as yet though it seems unlikely that a drop in hypothalamic temperature had occurred (195). Shivering is inhibited by hypoxia (152), CO_2 , and intravenous epinephrine (128). The effectiveness of shivering to compensate heat loss depends largely on the mass of shivering muscles, as, e.g., in the pigeon, where the big pectoral muscles are important heat sources in the cold (299). Horvath *et al.* (168) and Spurr *et al.* (297) have determined that in man shivering is about 11 per cent effective in protecting against total heat loss at -3°C .

VASCULAR REACTIONS

Cutaneous blood flow.—Further studies have been made on the influence of cold on blood flow in the dog's limb (132) and the isolated ear of the rabbit (98). Blood pressure and tone of small veins in man increase considerably in the cold (313, 318). Cold vasodilatation in the human finger does not change after application of antihistaminic substances (326) but decreases during general chilling (192). Cooper & Kerslake (69) believe the increase in heart frequency during skin heating to be reflex and not hemodynamic in origin, because it starts before vasodilatation occurs.

Does heating increase human skin blood flow by release of sympathetic vasoconstrictor tone or by stimulation of vasodilator fibres? An answer to this controversial question has been sought by plethysmographers using nerve blockade. Direct heating increases hand blood flow to about 50 per cent of the amount obtainable with a combination of direct and indirect heating (272). During indirect heating, Gaskell (114) and Roddie *et al.* (275) found no change in hand blood flow after nerve blocking. Thus, heat vasodilatation is brought about by release of constrictor tone alone. In the forearm, however, heat dilatation is abolished almost completely by nerve blockade, as Edholm *et al.* (89) have observed. Cholinergic mechanisms (274) and perhaps bradykinin formation from sweat gland stimulation (105, 106) might be involved in the active heat vasodilatation of the forearm skin.

Muscle blood flow.—Using a heated thermocouple introduced into the muscle, Barcroft *et al.* (22) noticed no change or a slight decrease of muscle blood flow in human limbs during indirect heating. This has been confirmed by Roddie *et al.* (271, 273, 276) and Edholm *et al.* (88) with different techniques, such as venous oxygen saturation and epinephrine electrophoresis of the skin. Söderberg (294) obtained similar results in the cat, in which local hypothalamic heating caused a decrease of muscle flow by 30 per cent. Clarke *et al.* (63) found an increased muscle flow on local cooling of the forearm. The increase was prevented by nerve blocking but not by sympathectomy. Determinations of muscular tone might decide whether this factor is involved in the increase in flow.

SWEATING AND EVAPORATION

Kuno's competent book on human perspiration (197) has appeared in a new edition. Evidence as to regional sweating patterns is given by Randall *et al.* (256), using direct sympathetic stimulation in man, and by Hertzman (162). Kerslake (193) found a close relationship between deep skin temperature and sweat rate. A series of measurements of Na, K, and urea concentrations in the sweat under various conditions has been carried out (58, 59, 214, 219, 267, 285). In wet heat the sweat clearance of urea may amount to values comparable with those of a kidney (58, 219). Schwartz & Thaysen (285) and Bulmer & Forwell (59) found a reciprocal linear relationship between Na concentration and sweat rate. At high rates, Na reaches a constant level equal to plasma concentration. The authors put forward the theory

that an isosmotic sweat production from the blood takes place with following reabsorption of Na limited to a maximum of about 50 m.eq./l.

Insensible perspiration.—This somewhat nebulous term includes diffusive water transport through the skin and low threshold sweat secretion. In the hand, a very low water output at high external vapour pressures and an input during immersion in water were observed by Craig (74). The correlation of insensible weight loss to vapor pressure gradient between skin and surroundings has aroused some discussion (48, 135, 188, 320). Kaufmann *et al.* (188) found a nonlinear relationship between vapor pressure gradient and total cutaneous weight loss in man below the so-called sweating threshold; water loss from low-rate sweating areas, such as palm and sole, was not taken into account. This factor is of considerable importance, as shown by a 60 per cent reduction of insensible perspiration from the hand after atropine injection (74). Having covered the low threshold sweating areas by plastic, Brebner *et al.* (48) found that the relationship between water transfer and vapor pressure gradient becomes linear. The same holds true for water loss at high temperatures after sweat gland activity has been suppressed by atropine (320).

NERVOUS MECHANISMS

Central mechanisms.—The present evidence from which to construct an engineer's blueprint of the heat-regulating system in terms of cybernetics and feedback mechanisms is far from adequate (3). Most physiologists agree that central body temperature is the leading factor in homeothermy. Glaser & Newling (121), finding the rectal temperature in man to be not constant, suggest that the essential mechanisms of temperature regulation in man are concerned with a constant thermal balance, not a constant deep body temperature. This theory cannot be considered conclusive, for certain types of automatic controllers (proportional action controllers) show an "offset" dependent on external disturbance; human temperature regulation probably represents such a system (254). On the other hand, thermal balance per se is not a feature with which to characterize homeothermy adequately. Even a physical heat source in the steady state has a "balance" of heat production and heat loss at any external temperature. If thermal balance were the essential mechanism, spontaneous rewarming from hypothermia, for instance, would be impossible (34).

Thermoregulatory mechanisms are activated from external as well as from internal temperature stimulation (76, 293). What "internal" or "central" temperature means is not clear; usually it means rectal temperature. As long as the respective temperatures are not measured directly, sweeping deductions should be avoided as to hypothalamic temperature changes and mysterious thermoreceptors somewhere in the body. Bligh (44) observed in the calf an onset of thermal polypnoea before blood temperature in the bicarotid trunk changed. In the anesthetized dog, panting is brought about by external and carotid heating as well (215).

There is no evidence in man for a spinal thermosensitive centre. Wyndham & Guttman (332), investigating a patient with a complete chronic transection lesion at C.6, noticed no sign of regulation against heat or cold, such as sweating, vasomotor reactions, and shivering, in the region innervated from the isolated spinal cord. Similar results were obtained by other workers (67, 258).

Birzis & Hemingway described a fairly discrete "shivering pathway" from the posterior hypothalamus to the spinal cord. The pyramidal tract, rubrospinal tract, and periventricular hypothalamic efferents seemed not involved, but bilateral section of the white columns of the thoracic cord abolished the shivering response in the hindlimbs (39). Electric stimulation of the medial part of the tuberal hypothalamus and of the shivering pathway in the midbrain elicited typical shivering (40). Impulse discharges were recorded along the shivering pathway, the frequency of single units ranging from 6 to 26 per sec. (41). Local hypothalamic heating inhibits gamma fibre activity, as found by von Euler & Söderberg (95). The hypothalamic thermoreceptive structures influence the activating relay system of the midbrain that controls wakefulness, and muscular tone as well. Vice versa, stimulation of the gamma motor system appears to set the body thermostat to a higher level, whereas inhibition of gamma motor activity lowers the level of temperature regulation (96).

Hypothalamic stimulation in unanesthetized animals.—Electric stimulation in the goat of the "heat loss centre" between anterior commissure and optic chiasma causes polypnoea, cutaneous vasodilatation, and inhibition of shivering (10). Andersson & Persson (11) were able to lower the rectal temperature in the goat to 29.5°C. by prolonged stimulation of the heat loss centre at room temperatures of -6°C. Cold defense reactions, such as shivering, peripheral vasoconstriction, and inhibition of panting, could be elicited by electric stimulation of the septal area, medial and dorsal to the heat loss centre (9).

Kundt *et al.* (195) implanted thermoneedles 0.4 mm. in diameter into the hypothalamus of cats over a period of some months. Unlike Forster & Ferguson (104), they established a close relationship between thermoregulatory vasomotor reactions and hypothalamic temperature fluctuations. Vasoconstriction produced in the ear by cooling the paws was followed by a rapid rise in hypothalamic temperature amounting to 0.6°C., the latency being only a few seconds, whereas warming caused vasodilatation and a subsequent temperature drop in the hypothalamus of 0.05 to 0.4°C. Rectal temperature changed in the same directions, but very slowly. Thus, under moderate conditions of heat and cold, hypothalamic temperature changes counteract the effects from peripheral afferents.

Ström's (301, 302) finding that hypothalamic cooling fails to produce vasoconstriction or shivering could not be confirmed in the unanesthetized cat by Kundt *et al.* (196) or by Hensel & Krüger (160) by use of a thin cooling tube chronically implanted into the hypothalamic region. Cooling by

1°C. or even less caused a marked vasoconstriction in the ear, as recorded by a heated thermocouple, the amount of vasoconstriction being closely related to the degree of cooling; shivering and a rise in rectal temperature were evoked too. Withdrawal of the cooling unit 5 mm. away from the hypothalamus extinguished these effects.

Thermoreceptors.—Most investigators of human temperature sense use well defined external stimuli but do not measure intracutaneous temperature patterns. Therefore, a great many of their deductions cannot be considered conclusive, e.g., some work on spatial summation (211) or the "thermopile" hypothesis (212). Concerning the latter, no specific refutation has been made as yet of electrophysiological work giving evidence that spatial gradients are not the adequate stimulus for thermoreceptors (56, 161, 242). In experiments with small thermal stimulators, a relationship between cross-sectional area of stimulus and thermal threshold may be a matter of physics, not of physiology (155).

Sinclair (290), discussing the anatomical basis for cutaneous sensations recently studied by the Oxford group led by Weddell, states that temperature and other sensations in man are elicited from skin areas where no organized endings of any kind can be found. The same holds true for the tongue of the cat; although mechanical and thermal impulse discharges are recorded from this area, Kantner (184) found nothing but a network of free nerve endings. As Sinclair admits, it remains possible that specific endings exist, but not in the sense of v. Frey's theory (107) of different organized endings. The difference must be too subtle for our present investigative methods.

It is generally agreed now that the cold receptors in the tongue exhibit a steady discharge frequency that depends on absolute temperature. Rapid cooling elicits an overshoot in frequency, whereas rapid warming causes a false start. The discharge is inhibited by hypoxia (156), increased CO₂ concentration (46, 81), and efferent stimulation of the lingual nerve (82), the stimulation probably causing vasomotor changes. Landgren (201, 202) recorded impulses from single cells in the cortical area of the tongue. About 4 per cent of the total number of cells reacted specifically to cold stimulation, whereas a higher percentage reacted to cold as well as to mechanical stimulation or even to a more complicated combination. The convergence of different qualities is ascribed to central nervous processes.

Cold receptor discharges from the external skin in dogs, cats, and rats have been reported (45, 225, 330). In the paw of the cat, Witt (330) found only very few specific cold receptors reacting like those of the tongue. A considerable number of nerve endings could be stimulated by cooling and pressure as well, but no warm receptor has been found as yet. Even in the trigeminal area, which is very sensitive to warmth, as proved by behavioral experiments in the awake cat, Boman (45) has observed as yet nothing but a cessation of cold impulses during warming. Iggo (173a) observed that single C fibre preparations responded to noxious stimuli on the skin as well as to light touch and to temperature changes.

Thermoreceptors in poikilotherms.—As any receptor discharge is influenced by temperature (155, 242, 243), the definition of a "thermoreceptor" is merely a matter of quantitative sensitivity. The ampullae of Lorenzini in the ray and dogfish, though reacting to mechanical stimulation (158, 244), are even more sensitive to temperature than the thermoreceptors in homeotherms. Hensel (157) found that the isolated ampulla contains a uniform population of "cold" receptors, with a steady discharge between 3 and 34°C. and a maximum at about 20°C. The reactions to cooling and warming, as well as to changes in O₂ and CO₂ pressure, are the same as those found in the cold receptors of the tongue (159). Pure "warm" receptors are the nerve endings in the thin membrane of the facial pit in pit vipers. Bullock & Diecke (56), in an elaborate study, found an irregular steady discharge and an exceedingly high overshoot sensitivity to warming, whereas cooling caused a false start. The receptors are able to detect infrared radiation from warm blooded animals. Even in these organs, only flattened palmate nerve structures with free endings could be seen by Bullock & Fox (57).

HEAT STRESS

Animals.—Lee (207) discusses the practical importance of heat tolerance in cattle. Heat tolerance of guinea pigs varies inversely with body size, probably an effect of surface-volume ratio (327). Donhoffer *et al.* (83) conclude from experiments with hypothalamic lesions that the metabolic increase in the hyperthermic rat is of central nervous origin and not a direct temperature effect on peripheral tissue metabolism. At high environmental temperatures inorganic phosphate in plasma and urine of dogs decreases, possibly as a result of polypnoeic alkalosis (183). A blood sugar drop of 16 to 22 per cent might be caused by metabolic factors since no glucosuria occurred (182). Acute hyperthermia of 42.5°C. increases plasma levels of 17-hydroxycorticosteroids in the dog from 4 to 15 µg. per cent or even more (23). During heating of animals to a rectal temperature of 44 or 45°C., Richards & Egdahl (262) observed circulatory failure with a drop in 17-hydroxycorticosteroid excretion. Hypophysectomy abolishes the increase of corticoids during heating. On exposure to heat, water excretion of normal rats, but not that of diabetes insipidus rats, was markedly reduced (177).

Man.—The effective temperature scale has not proved satisfactory to assess the physiological severity of hot climates, for thermal comfort is not equal to physiological thermal stress. Lind & Hellon (216), defining physiological heat stress by "predicted 4-hr. sweat rate" (P₄SR), found the P₄SR-scale to be adequate under various hot conditions. Investigations on men working in the heat have been continued (199, 217, 230, 251, 322). Ladell (199) found a lower sweat rate after replacing drinking water by saline. Sweating is independent of the amount of dehydration until water deficit exceeds 2.5 l. Pearcy *et al.* (251) have demonstrated that dehydration reduces sweat secretion even if thermoregulation fails and body temperature rises. Petrunj (252) found a cutaneous oxygen absorption of 193 ml. per hour in

the resting subject; during work and heat, the absorption increased to 730 ml. per hour.

Exposure to 41°C. room temperature shifts the Na/K ratio in urine from 1.5 to 0.4 (221); this might be in connection with a higher output of aldosterone. Hellman *et al.* (149) observed an increased urinary excretion of aldosterone during heat stress but no change in the excreted 17-hydroxycorticosteroids. No change, or, in some cases, a slight increase of plasma ACTH occurs (136) whereas 17-ketosteroid excretion drops by 50 per cent during work in hot atmospheres (266).

COLD STRESS

Animals.—The mechanism of "restraint" or "emotional" hypothermia in rodents (27) remains a matter for conjecture. Bartlett *et al.* (26) found the temperature drop in restrained rats to be more pronounced at higher muscular activity. A warning to experimenters who use restrained animals at low room temperatures comes from Wilber & Robinson (329), stressing that in the cold every route of heat loss might become significant. Adjustment of food intake to cold has been studied in pigeons (298). Using C¹⁴ incorporation into fatty acids, Masoro *et al.* (226, 228) found a high lipogenesis from carbohydrates in the cold even during weight loss of the animal.

Many problems remain to be solved concerning the role of endocrine glands in acute cold stress. The stimulation of adrenocortical function is apparently transient in nature and, moreover, varies widely with species (47). Egdahl & Richards (90), exposing dogs for some hours to extreme cold without hypothermia, found that the increased steroid output from the lumbo-adrenal vein returned to normal values within 1 to 3 hr. despite continued cold stress. This effect cannot be explained by adrenal exhaustion, since ACTH injection evoked an immediate rise in steroid output.

Cold of 10°C. increases I¹³¹ output from the thyroid gland of the rat, but at 2°C. a depression of thyroid activity takes place (51). The increased thyroid activity in the cold is depressed by posterior pituitary hormones (16). Bartlett (25) demonstrated that in restrained rats the I¹³¹ uptake in the cold is higher than in normal animals. Thyroxine administration was found to increase basal metabolism in the rat at 29°C. but not to stimulate the metabolic response to cold (12). Thyroidectomy shifts the critical temperature of the rat from 29 to 33°C. (210). Significant decreases of blood protein-bound iodine (187) and of the epinephrine-potentiating effect of thyroxine (309) have been observed in the cold.

Man.—Cold tolerance is an important factor in hazards to shipwrecked persons as proved by a review of the cases during World War II. The mortality was from 20 to 30 per cent at water temperatures of 5°C., and decreased to 1 per cent at 20°C. (233). Studies on human performance in the cold have shown that manual dexterity (310), tactile discrimination (239), and visual-motor performance (311) are seriously impaired. Ralli *et al.* (255) have tested effects of vitamin supplementation on the reaction to acute

cold stress. Kreider & Buskirk (194) reported that thermal comfort of men sleeping in the cold is considerably improved by supplementary feeding. Six hundred or 1200 kcal. given before sleeping increased rectal and peripheral temperatures as well as metabolic rate.

ACCLIMATIZATION

The term "acclimatization" or "acclimation" is widely used by physiologists, whereas "adaptation" is claimed by physiologists as well as by biologists for at least three quite different processes. Hart (148) stresses the necessity for differentiating between natural and laboratory experience; for the latter the terms "acclimation" or "conditioning" are suggested. In a comparative study Adolph (2) finds more adaptations to be specific than non-specific to diverse stressors, such as cold, low oxygen pressure, etc., and questions on the basis of these findings the concept of a general nonspecific adaptation syndrome. Adaptations are thought to be superimpositions or modifications of more stable regulating mechanisms. The basic processes inducing acclimatization remain obscure. What is the role of thermal receptors? What is the nature of peripheral acclimatization? Which central nervous mechanisms are involved? None of these questions can be answered at present, leaving a wide field for future research.

Some additional data on animals living in hot or cold climates are available. Small mammals in the tropics have critical temperatures of about 27°C., whereas those of birds range between 24 and 20°C. (93). Irving *et al.* (176) proved that the melting points of fat in arctic animals, being 10°C. in the distal and 40 to 50°C. in the proximal parts of the extremities, are not determined by acclimatization. Even in man and in tropical animals a similar pattern is found. Camels are able to tolerate 17 days in the heat without water supply. As demonstrated by Schmidt-Nielsen *et al.* (279), this is achieved by a dehydration tolerance amounting to 30 per cent, low water loss by urine and feces as well as by evaporation, and tolerance to high body temperatures.

Wilber (328) critically discusses Bergmann's and Allen's rules and rejects any causal role for the origin of human types (284). As he justly says, in man the ecological forces supposed to be acting are not doing so: Eskimos were not cold, the skinny aboriginal Australians were. Behavioral and technological as well as physiological adjustments are possible in man without gross morphological changes.

Heat acclimatization.—Heat acclimatization in mice increases anoxic resistance (163). The melting point of body fat, but not of cutaneous fat, rises with high environmental temperature (336). Robinson & MacFarlane (265) found a doubling of antidiuretic substances in the heat acclimatized rat. Histological changes in the posterior pituitary lobes and in hypothalamic nuclei were observed by Ueno (314) in rats exposed to heat and cold. In the chicken, heat adjustment of body temperature requires three to five days, whereas respiratory frequency is acclimatized after eight to ten days (164).

Acclimatization to heat has been studied chiefly in man. Evidence is given that no essential difference in heat tolerance exists between Bushmen and acclimatized Europeans (334). Most of heat adjustment is achieved within a few days, the process being delayed by atropine (75), whereas loss of acclimatization occurs after six days (333). A reduction of 17-ketosteroid output by 23.7 per cent has been found by Robinson *et al.* (264) in the spring as compared with the summer. Hand blood flow apparently does not change during repeated heat exposure (325). The reduced salivation in the heat returns to normal during heat acclimatization (291). Changes in blood volume are still a matter of discussion. Bass *et al.* (28), in a reinvestigation under standard conditions, found plasma, blood, and circulating red cell volume not to be altered by heat acclimatization for three weeks. The findings of increased blood volumes in the summer and during sedentary living in hot climates (29) might be due to continuous living in the heat, not to the acclimatization process per se.

Cold acclimatization in animals.—The climatic and temperature influences on the energetics of homeotherms have been recently discussed by Hart (148) in an excellent review. Only a few additional remarks will be given here. Changes of metabolism and overall insulation are the predominant features of adjustment to cold in animals. Apparently not all changes induced by prolonged cold are beneficial, e.g., coronary lipide deposition in the rat (287). Cold acclimatization reduces rapid reactions to acute cold stress, e.g., oxidation of C¹⁴-glucose (78) or incorporation of P³² in the adrenals (246). Masoro *et al.* (227) have found that hepatic lipogenesis is depressed during prolonged cold exposure. According to Hannon (141, 142), respiration of liver tissue from rats kept in the cold for four weeks was increased; no increase was found after nine weeks of cold acclimatization. Anaerobic glycolysis, however, decreased continuously during cold exposure. In the monkey, Dugal & Fortier (87) did not observe an effect of ascorbic acid on the respiratory quotient during six months of mild cold exposure. Further studies are concerned with changes in mast cells (206), blood clotting and electrophoretic properties of plasma (308), and electrolyte metabolism (20) during prolonged cold. Covino & Beavers (70) found the incidence of ventricular fibrillation in hypothermia to drop from 96 to 9 per cent in dogs which had been kept previously for one to four weeks in the cold; deacclimatization is accomplished after five days.

Cold acclimatization in man.—Unlike the dramatic reactions in heat acclimatization, the physiological changes in man during prolonged cold exposure are rather small, being apparently more important for thermal comfort than for regulation of body temperature against cold. Living in severe cold is a matter of "cultivation", of behavioral and technical adjustments rather than of physiological acclimatization (268).

Several reports on caloric requirements of men living and working in the Arctic have been published (181, 204, 229, 321), the optimum requirement ranging between 3000 and 4200 kcal. a day. Living in a cold climate has little or no effect on the diurnal pattern of rectal temperature (173). Sea-



sonal variations of basal metabolism and protein-bound iodine in Japanese subjects have been ascribed to changes of thyroid activity (249). Rodahl & Bang (269), however, using I^{131} uptake, did not find any sign of increased thyroid activity in men exposed to cold.

The Eskimo, as well as the Lapp, lives in an almost tropical microclimate. As Rodahl (268) points out, the cold adjustment of Eskimos merely consists in avoiding the cold. Scholander *et al.* (283) found the critical temperatures in Lapps to be the same as in man living in moderate climates (94). Physiological differences between Eskimos, white men, and Negroes might be caused by racial differences rather than acclimatization, perhaps with the exception of higher finger temperatures in the Eskimo during cold stress (1, 235, 260). Adams & Covino (1) have confirmed that basal metabolic rates of Eskimos are about 30 per cent higher than those of Caucasians and Negroes; the metabolic increase to a standard cold stress, however, is the same in Eskimos and Caucasians whereas the response of the Negro group is less. According to the higher basal metabolism, the sweat rate of Eskimos in the heat is higher than in the control group (270). Differences in plasma volume (31) and blood levels of adenine nucleotides between Eskimos, Caucasians, and Negroes have been reported (140).

Prolonged severe cold stress is practically without influence on basal metabolic rate (172, 281); in the cold, however, metabolism, as well as cutaneous blood flow, is significantly higher in cold acclimatized subjects than in controls (92, 281). Scholander *et al.* (281) found that foot temperature of acclimatized white men resting in the cold was 32°C., control values being as low as 18°C. The reduced thermal insulation of acclimatized persons is compensated by a higher metabolic rate. A quite different mechanism of cold adjustment, which is physiologically more effective, has been found by Scholander *et al.* (280a) in the naked Australian aborigines. Their thermal insulation in the cold is higher than that of unacclimatized whites. At air temperatures frequently dropping to 0°C., the natives sleep through the night with normal resting heat production, with their foot temperature dropping to 12°C. Thus, cold acclimatization in man apparently aims to reduce thermal discomfort and peripheral cold injury, at the price of a waste of heat. The results concerning rectal temperature changes during cold acclimatization are not consistent (172, 203, 281); if existing at all, the changes are very small. The overshooting metabolic response to acute cold stress seems to be diminished by cold acclimatization. As LeBlanc (203) says, the body acquires "more confidence in itself." In the reviewer's opinion, psychological factors should be taken into account, for acute cold stress is combined with nonspecific affective reactions causing per se peripheral vasoconstriction and increased muscular tone (124). It remains uncertain whether similar factors are involved in the changes of the cold pressor test found by LeBlanc & Rosenberg (205) during repeated local cold exposure. A decrease in latency of cold vasodilatation, as observed in the same experiments, gives further evidence for local cold acclimatization. Glaser & Whittow (122) reported that cold pain was reduced and blood pressure and pulse rate rose less after

repeated immersion of the hand in water of 4°C. This adaptation to localized cooling persisted for one day in a warm environment and was confined to the hand which had been repeatedly cooled. The authors explain their findings on the basis of central nervous processes.

HYPOTHERMIA

This subject has been discussed at length in two conferences (64, 86) and a recent review (292a). Hypothermia has now lost somewhat of its practical interest for cardiac surgery, since extracorporeal circulation is increasingly used. The so-called "artificial hibernation" or moderate pharmacological hypothermia has not been included in this review, for this procedure does not suppress thermoregulation in the dog (200), nor has it any "histoplegic" effect (315). Hibernators are treated only with respect to hypothermia. Concerning hibernational sleep, which is a normothermic regulation at a low level, Eisentraut's book (91) and the last review by Kayser (189) may be consulted.

Heat exchanges.—Behmann & Bontke (35) developed a method for intravascular cooling with automatic control of rectal temperature within 0.1°C. Even deep anesthesia does not abolish nervous thermoregulation completely (34, 36). Intravascular cooling of dogs under light anesthesia causes shivering below an aortic temperature of 36.3°C. without stimulation of peripheral cold receptors (33). Internal temperature gradients have been measured in the dog (295) and in man (68, 303) during hypothermia. Werner *et al.* (324) have observed spontaneous rewarming from a rectal temperature of 25°C. in the unanesthetized curarized dog, thus demonstrating the importance of chemical thermogenesis. The critical body temperature at which spontaneous rewarming from hypothermia occurs is much lower in the hibernator than in the homeotherm, as Adolph & Richmond (4) have demonstrated; rewarming rates of hibernators are also much higher.

Respiration, circulation, body fluids.—Arterial-alveolar O_2 and CO_2 gradients have been measured during hypothermia in the dog (7, 250, 288) and in man (288). Gas exchanges are reduced in hypothermia but still sufficient for the lower metabolism. Albers *et al.* (7) suggest the existence of functional atelectases in the hypothermic dog; the diffusion factor for O_2 turned out to be 0.44 at 38°C. and 0.26 ml. min.⁻¹ kg.⁻¹ mm. Hg⁻¹ at 20°C. Further evidence is given (6) that no hypoxia occurs during hypothermia; even arterial hypoxemia as low as 17 mm. Hg P_{O_2} has no influence on the lethal temperature.

Brendel *et al.* (49, 50) have observed responses to carotid sinus stimulation, CO_2 inhalation, and stimulation of cutaneous cold receptors at rectal temperatures of 20°C. Covino & Beavers (71) found an increased blood flow in the hind limb of the hypothermic dog; it is suggested that in moderate cooling the increased flow is due to cholinergic vasodilator fibres, whereas lower temperatures act directly upon the blood vessels. During deep hypothermia, Lynch & Adolph (220) did not notice a change in diameter of small vessels; blood flow was reduced by increased viscosity.

Acid-base balance during hypothermia is greatly dependent on anesthesia and respiration (17, 120). Metabolic acidosis in man can be prevented by hyperventilation, provided that no additional stress, e.g., shivering, occurs (154).

Heart.—Much research work has been focused on the hypothermic heart, the main problems still being ventricular fibrillation and cardiac efficiency. Angelakos (13) demonstrated statistically that the terminal temperatures in dogs are independent of either ventricular fibrillation or asystole as the terminal events. Although considerable practical progress has been made in controlling ventricular fibrillation during hypothermia (32, 66, 73, 198, 261, 263), the basic cause of hypothermic cardiac arrhythmias is still unknown. Covino & Beavers (72) have found a fivefold decrease of ventricular fibrillary threshold at 22°C. but a constant basic ventricular threshold. Changes in refractoriness are obviously not the cause of fibrillation (15). Important factors in inducing cardiac arrhythmias are acidosis (73), potassium loss from the myocardium (126, 232), hypersensitivity to sympathetic stimulation (113, 289) and to calcium (14). Hannon & Covino (143) suggest that changes in cellular metabolism of the myocardium might be the basic cause of fibrillation, but no biochemical evidence for this has been found as yet (143, 178).

As has been confirmed by several investigators, the hypothermic heart does not undergo undue stress. In the heart-lung preparation the working capacity is higher than necessary for the hypothermic organism (19, 259). Badeer & Khachadurian (19) proved that the higher mechanical efficiency of the heart during hypothermia results from cold per se and not from bradycardia. Hansen *et al.* (144) could not observe any decrease of cardiac efficiency in the dog when rectal temperature was decreased from 38 to 23.5°C. Coronary O₂ utilization remained constant, whereas coronary blood flow, O₂ consumption of the left ventricle, and total O₂ consumption dropped proportionally to temperature, reaching about 25 per cent of the initial value at 23.5°C. Fisher *et al.* (103) found the first signs of cardiac failure to occur after 14 hr. at 23°C. Gollan & Nelson (125), using extracorporeal circulation in combination with general hypothermia of 20°C. in the dog, achieved an anoxic tolerance of the resting heart of 1 hr.

Nervous system.—The spontaneous cortical activity in the rat stops at 16 to 18°C., whereas evoked potentials are noticed even at 13°C. The depression in nervous function is preceded by a transient stage of hyperexcitability (30). Oxygen consumption, blood flow, and occlusion tolerance of the brain have been studied in monkeys at 25°C.; a tolerance limit of 12 min. has been found (38, 234). Localized cerebral cooling in the dog by means of an extracorporeal circulation has practically the same effects as has general hypothermia (331). Malmjeac *et al.* (222) find that, in dogs and monkeys cooled to about 18°C. for a short time, cortical recovery as studied by conditioned reflexes needs three to eight days, this time being decreased by epinephrine infusion during hypothermia. Epileptiform activity (21) and effects of vagal stimulation (337) are diminished at low temperatures. During the phase of hyperexcitability between 35 and 25°C., a spreading of synaptic transmission

to the surrounding neurons takes place (304). In the hibernator rewarming from hypothermia, the first electrical activity is seen in the midbrain at temperatures of 6 to 8°C. This holds also for the arousal from natural hibernation; however, other parts of the brain are active in hibernation sleep at lower temperatures than in hypothermia (257).

Miscellaneous.—Whether hypothermia evokes a general stress response depends largely on anesthesia; barbiturates appear to inhibit stress (100, 278). No evidence for tissue damage is found generally in hypothermia (101); however, necrotic foci in the heart have been described by Sarajas (277). Fructose metabolism (335), hepatic oxygen consumption (97, 102), and protein formation in the liver (18) have been investigated in man and animals during hypothermia. Intestinal absorption below 19°C. appears to be a filtration independent of osmotic pressure and nature of substances absorbed (119). Further studies have been concerned with renal function. Renal circulation and glomerular filtration rate are greatly reduced during hypothermia (42, 166, 240). Also a reduction of the reabsorptive and secretory tubular functions takes place (166, 286). The tubular maximum of *p*-aminohippuric acid has been confirmed to decrease considerably at low temperatures (42, 240).

Deep hypothermia and suspended animation.—Cooling of homeotherms to body temperatures of about 0°C. with cardiac standstill may be followed by resuscitation, provided that rewarming starts within 1 to 2 hr. This was confirmed in further experiments, e.g., in the monkey, where Niazi & Lewis (245) observed complete recovery after 2 hr. of cardiac asystole at rectal temperatures of 4 to 9°C. It is not known as yet why homeotherms cannot survive severe hypothermia for more than this short period. The limiting time factor is apparently independent of temperature in the range below 15°C. (5).

Reanimation of ice-cold rats is achieved by artificial respiration and simple irradiation with a bench lamp (122a). In these experiments Goldzweig & Smith (123) found a severely reduced fertility for eight weeks. Smith (292) succeeded in resuscitating golden hamsters from cooling to -5°C. internal body temperature; recovery is possible even if 50 per cent of the body water had been frozen (218).

Even at complete standstill of the hypothermic heart, the pacemaker fibres continue to discharge. The link between pacemaker and auricle, broken by cooling, can be restored by acetylcholine (223, 224). No changes in membrane and action potentials occur in the dog's heart, when it has been kept previously at 1 to 3°C. for 72 hr. (231). The changes in heart performance following resuscitation from deep hypothermia of 15°C. for 1 to 3 hr. have been studied by Giaja & Radulović (116). In the posthypothermic state, the anoxic resistance of the heart shows a fourfold increase; also the working capacity is doubled (118). The profound posthypothermic changes are further demonstrated by an increased survival time of the isolated heart taken from rats in deep hypothermia (117). The temperature for cardiac standstill in hypothermia is different in hibernators and homeotherms. Kayser and co-

workers (190, 191) found asystole to occur at 0°C. in hibernators and at 15°C. in homeotherms. No differences, however, could be detected between the two groups in the temperature coefficients of heart frequency or of diverse parts of the electrocardiogram.

COLD INJURY

This subject has been treated in the Fourth Macy Conference on cold injury (99) and in two reviews by Meryman (236, 237). Many factors might be involved in local cold injury, such as vasoconstriction, thrombosis, nerve lesions, ice formation, and dissociation of anabolic and catabolic processes at low temperature (145). Present research is progressing mainly along two lines: vascular responses and tissue freezing.

Freezing of tissues.—The freezing point of living tissue has been found by Pichotka *et al.* (253) to be $-1.08 \pm 0.04^\circ\text{C}$., thus suggesting that intracellular fluid is not isotonic with blood plasma. The mechanism maintaining the higher intracellular osmotic pressure is not known as yet; possibly it is an active metabolic water transport out of the cell. However, the freezing point of organized tissues is no suitable measure of osmotic pressure, as Kuhn (194a) has shown. Local cooling leads to an increased water uptake and swelling of the cells (5, 65). According to Scholander *et al.* (280) freezing of arctic fishes is prevented either by supercooling and avoidance of ice seeding or by an increased osmotic pressure. In intertidal animals, however, 80 per cent of body water can freeze without damage of the organism (185).

The deleterious factor in tissue freezing is increase in concentration of electrolytes and other substances rather than mechanical damage by ice formation (236, 237). Clinical frostbite usually occurs at moderate rates of cooling, leading to extracellular ice formation, whereas intracellular freezing is only seen during rapid cooling. In this respect, laboratory experiments in animals have been criticized by Meryman (237) as being not comparable at all with frostbite in man. Fuhrman (110) has shown that oxygen consumption and anaerobic glycolysis of isolated rat tissue are disturbed neither after prolonged cooling at -0.4°C . without freezing, nor after supercooling, but are greatly reduced after freezing. Rapid thawing in water of 42°C . is considered the best treatment of frostbite, at least in animal experiments (111, 112, 237, 306). Brief freezing or burning preceding a standard frostbite reduces tissue loss (109).

Vascular reactions.—Vascular responses to cold injury in the cheek pouch of golden hamsters have been investigated by Sullivan and co-workers (305, 306, 307). After thawing of the tissue, the blood flow is increased initially; later a gradual decline in rate of flow occurs, with arteriolar constriction, thrombosis, and stasis. Inositol causes a greater edema but reduces stasis and tissue loss. Imig *et al.* (174) noticed an increase in blood flow above the initial value in the dog's hind limb after thawing, whereas Hardenbergh & Bamberg (145) found the flow after thawing to be slightly reduced or normal. As long as freezing did not occur, reactions to epinephrine and acetylcholine remained unchanged during cooling.

ONTOGENY OF THERMOREGULATION

An important comparative survey on the ontogeny of physiological regulations in the rat is given by Adolph (3). More attention should be paid to the question whether postnatal changes of thermoregulation are congenital patterns or caused by external conditions, such as temperature or birth per se. The metabolic thermoregulation in piglets is very unsatisfactory within the first six days and becomes nearly perfect around the twentieth day after birth (165). A somewhat more developed thermogenesis has been reported by Gelineo (115) as a result of experiments in newborn dogs. Even though temperature regulation is still very imperfect, signs of homeothermic behavior of metabolism can be seen one day after birth. Thyroidectomy in young rats has no influence on the development of thermoregulation within 14 days after birth but depresses rectal temperature and metabolic rise in the cold after 18 days (133, 134).

Development of thermoregulation in man.—It is surprising enough that, despite an immense amount of work in newborn animals and in adult man, very little is known about the ontogeny of thermoregulation in man. Brück *et al.* (52, 53, 54), using a small climatic chamber (55), have followed up metabolic and vasomotor thermoregulatory responses in premature and full-term newborn infants from the very first hour until 14 days after birth. The minimal metabolic rate at a room temperature of 33°C. is 1.4 to 1.6 kcal. kg.⁻¹ hr.⁻¹ in premature as well as in full-term babies, and remains constant for the whole period of time. In full-term infants the metabolic rise in response to a standard cold stress of 23°C. amounts to 100 per cent at birth and increases to about 170 per cent after eight days (52). At birth an almost maximal cutaneous vasoconstriction is seen at a room temperature of 23°C, no dilatation occurring on warming, whereas at the end of the first week a considerable dilatation takes place at 35°C. (53).

Similar experiments have been carried out in premature newborn infants of 1550 to 2050 gm. (52). They show the same postnatal pattern of development of vasomotor and metabolic responses, the latter being 30 to 50 per cent smaller than in full-term infants; but, for example, the metabolic rise on cooling of a premature infant two weeks after birth is even higher than that of a full-term infant at birth. From these facts it is possible to conclude that the pattern of postnatal development of human temperature regulation is greatly dependent on birth per se and external conditions, or, in other words, is a matter of postnatal rather than of conceptional age. It seems necessary to reconsider, on this basis, the material on the ontogeny of thermoregulation in animals.

Aging.—Experiments in animals (171) and in man have shown a decrease in thermoregulatory responses with age. Hellon & Lind (150) found no significant difference in the number of active thermal sweat glands in the heat but a doubling of time for onset of sweating in the older men. In the latter, forearm blood flow in the heat was higher (151). Horvath *et al.* (167) compared the reactions to a cold stress of 10°C. in two age groups of men averaging 25.5 and 64 years respectively. In the old men, no subjective dis-

comfort, shivering, metabolic rise, or increased respiratory volume could be observed; peripheral vasoconstriction was less marked than in the younger group, and rectal temperature dropped by 0.3°C. The young men showed all signs of cold defense, including subjective discomfort, and kept their rectal temperature constant. A diminished cold vasodilation in old people has been noticed by Spurr *et al.* (296).

LITERATURE CITED

1. Adams, T., and Covino, B. G., *J. Appl. Physiol.*, **12**, 9 (1958)
2. Adolph, E. F., *Am. J. Physiol.*, **184**, 18 (1956)
3. Adolph, E. F., *Quart. Rev. Biol.*, **32**, 89 (1957)
4. Adolph, E. F., and Richmond, J., *J. Appl. Physiol.*, **8**, 48 (1955/56)
5. Adolph, E. F., and Richmond, J., *Am. J. Physiol.*, **87**, 437 (1956)
6. Albers, C., Brendel, W., Hardewig, A., and Usinger, W., *Arch. ges. Physiol.*, **266**, 373 (1958)
7. Albers, C., Brendel, W., Hardewig, A., and Usinger, W., *Arch. ges. Physiol.*, **266**, 394 (1958)
8. Alpen, E. L., Butler, C. P., Martin, S. B., and Davis, A. K., *J. Appl. Physiol.*, **8**, 399 (1955/56)
9. Andersson, B., *Acta Physiol. Scand.*, **41**, 90 (1957)
10. Andersson, B., Grant, R., and Larsson, S., *Acta Physiol. Scand.*, **37**, 261 (1956)
11. Andersson, B., and Persson, N., *Acta Physiol. Scand.*, **41**, 277 (1957)
12. Andik, I., Nagy, L., and Tóth, I., *Acta Physiol. Acad. Sci. Hung.*, **8**, 399 (1955)
13. Angelakos, E. T., *Proc. Soc. Exptl. Biol. Med.*, **97**, 107 (1958)
14. Angelakos, E. T., Deutsch, S., and Williams, L., *Circulation Research*, **5**, 196 (1957)
15. Angelakos, E. T., Laforet, E. G., and Hegnauer, A. H., *Am. J. Physiol.*, **189**, 591 (1957)
16. Arimura, A., Takagi, Y., and Ueno, T., *Japan. J. Physiol.*, **6**, 284 (1956)
17. Axelrod, D. R., and Bass, D. E., *Am. J. Physiol.*, **186**, 31 (1956)
18. Babskaya, Y. E., *Dokl. Akad. Nauk S.S.S.R.*, **114**, 598 (1957)
19. Badeer, H., and Khachadurian, A., *Am. J. Physiol.*, **192**, 331 (1958)
20. Baker, D. G., and Sellers, E. A., *Can. J. Biochem.*, **8**, 631 (1957)
21. Baldwin, M., Frost, L. L., Wood, C. D., and Lewis, S. A., *Science*, **124**, 931 (1956)
22. Barcroft, H., Bock, K. D., Hensel, H., and Kitchin, A. H., *Arch. ges. Physiol.*, **261**, 199 (1955)
23. Barlow, G., Agersborg, H. P., Jr., and Keys, H. E., *Proc. Soc. Exptl. Biol. Med.*, **93**, 280 (1956)
24. Bartholomew, G. A., and Wilke, F., *J. Mammal.*, **37**, 327 (1956)
25. Bartlett, R. G., Jr., *Proc. Soc. Exptl. Biol. Med.*, **94**, 654 (1957)
26. Bartlett, R. G., Jr., Bohr, V. C., Foster, G. L., Miller, M. A., and Helmendach, R. H., *Proc. Soc. Exptl. Biol. Med.*, **92**, 457 (1956)
27. Bartlett, R. G., Jr., Bohr, V. C., and Helmendach, R. H., *Physiol. Zool.*, **29**, 256 (1956)
28. Bass, D. E., Buskirk, E. R., Iampietro, P. F., and Mager, M., *J. Appl. Physiol.*, **12**, 186 (1958)
29. Bass, D. E., and Henschel, A., *Physiol. Revs.*, **36**, 128 (1956)
30. Battista, A. F., *Am. J. Physiol.*, **191**, 209 (1957)

31. Baugh, C. W., Bird, G. S., Brown, G. M., Lennox, C. S., and Semple, R. E., *J. Physiol. (London)*, **140**, 347 (1958)
32. Beavers, W. R., and Covino, B. G., *Arch. Surg.*, **75**, 776 (1957)
33. Behmann, F. W., *Arch. ges. Physiol.*, **263**, 166 (1956/57)
34. Behmann, F. W., *Arch. ges. Physiol.*, **266**, 422 (1958)
35. Behmann, F. W., and Bontke, E., *Arch. ges. Physiol.*, **263**, 145 (1956/57)
36. Behmann, F. W., and Bontke, E., *Arch. ges. Physiol.*, **266**, 408 (1958)
37. Benzinger, T. H., Huebscher, R. G., Minard, D., and Kitzinger, Ch., *J. Appl. Physiol.*, **12**, S 1 (1958)
38. Bering, E. A., Jr., Taren, J. A., McMurrey, J. D., and Bernhard, W. F., *Surg., Gynecol. Obstet.*, **102**, 134 (1956)
39. Birzis, L., and Hemingway, A., *J. Neurophysiol.*, **19**, 37 (1956)
40. Birzis, L., and Hemingway, A., *J. Neurophysiol.*, **20**, 91 (1957)
41. Birzis, L., and Hemingway, A., *J. Neurophysiol.*, **20**, 156 (1957)
42. Blatteis, C. M., and Horvath, S. M., *Am. J. Physiol.*, **192**, 357 (1958)
43. Bligh, J., *J. Physiol. (London)*, **136**, 404 (1957)
44. Bligh, J., *J. Physiol. (London)*, **136**, 413 (1957)
45. Boman, K., *Acta Physiol. Scand.*, **44**, Suppl. 149, 79 pp. (1958)
46. Boman, K., Hensel, H., and Witt, I., *Arch. ges. Physiol.*, **264**, 107 (1957)
47. Boulouard, R., *Compt. rend. soc. biol.*, **151**, 913 (1957)
48. Brebner, D. F., Kerslake, D. McK., and Waddell, J. L., *J. Physiol. (London)*, **132**, 225 (1956)
49. Brendel, W., Albers, C., and Usinger, W., *Arch. ges. Physiol.*, **266**, 341 (1958)
50. Brendel, W., Albers, C., and Usinger, W., *Arch. ges. Physiol.*, **266**, 357 (1958)
51. Brown-Grant, K., *J. Physiol. (London)*, **131**, 52 (1956)
52. Brück, K., *Arch. ges. Physiol.*, **268**, 7 (1958)
53. Brück, K., Brück, M., and Lemtis, H., *Arch. ges. Physiol.*, **266**, 518 (1958)
54. Brück, K., Brück, M., and Lemtis, H., *Arch. ges. Physiol.*, **267**, 382 (1958)
55. Brück, K., and Hensel, H., *Arch. ges. Physiol.*, **266**, 556 (1958)
56. Bullock, T. H., and Diecke, F. P. J., *J. Physiol. (London)*, **134**, 47 (1956)
57. Bullock, T. H., and Fox, W., *Quart. J. Microscop. Sci.*, **98**, 219 (1957)
58. Bulmer, M. G., *J. Physiol. (London)*, **137**, 261 (1957)
59. Bulmer, M. G., and Forwell, G. D., *J. Physiol. (London)*, **132**, 115 (1956)
60. Burton, A. C., *Rev. can. biol.*, **16**, 293 (1957)
61. Burton, A. C., and Edholm, O. G., *Man in a Cold Environment* (Edward Arnold, Publishers, Ltd., London, Engl., 273 pp., 1955)
62. Burton, A. C., Snyder, R. A., and Leach, W. G., *J. Appl. Physiol.*, **8**, 269 (1955/56)
63. Clarke, R. S. J., Hellon, R. F., and Lind, A. R., *Clin. Sci.*, **17**, 165 (1958)
64. Colloques Nationaux du Centre National de la Recherche Scientifique, *Arch. sci. physiol.*, **9**, C 3 (1955)
65. Conway, E. J., and Geoghegan, H., *J. Physiol. (London)*, **130**, 438 (1955)
66. Cookson, B. A., and DiPalma, J. R., *Am. J. Physiol.*, **188**, 274 (1957)
67. Cooper, K. E., Ferres, H. M., and Guttmann, L., *J. Physiol. (London)*, **136**, 547 (1957)
68. Cooper, K. E., and Kenyon, J. R., *Brit. J. Surg.*, **46**, 616 (1957)
69. Cooper, K. E., and Kerslake, D. McK., *Clin. Sci.*, **14**, 125 (1955)
70. Covino, B. G., and Beavers, W. R., *Am. J. Physiol.*, **191**, 153 (1957)
71. Covino, B. G., and Beavers, W. R., *J. Appl. Physiol.*, **10**, 146 (1957)
72. Covino, B. G., and Beavers, W. R., *Proc. Soc. Exptl. Biol. Med.*, **95**, 631 (1957)

73. Covino, B. G., and Hegnauer, A. H., *Surgery*, **40**, 475 (1956)
74. Craig, F. N., *J. Appl. Physiol.*, **8**, 473 (1955/56)
75. Cullumbine, H., and Miles, S., *Quart. J. Exptl. Physiol.*, **41**, 162 (1956)
76. Davis, T. R. A., and Mayer, J., *Am. J. Physiol.*, **181**, 669 (1955)
77. Davis, T. R. A., and Mayer, J., *Am. J. Physiol.*, **181**, 675 (1955)
78. Depocas, F., Macleod, G. K., and Hart, J. S., *Rev. can. biol.*, **16**, 83 (1957)
79. Derksen, W. L., Murtha, T. D., and Monahan, T. I., *J. Appl. Physiol.*, **11**, 205 (1957)
80. Dimitroff, J. M., Kuppenheim, H. F., Graham, I. C., and McKeehan, C. W., *J. Appl. Physiol.*, **8**, 532 (1955/56)
81. Dodt, E., *Arch. ges. Physiol.*, **263**, 188 (1956/57)
82. Dodt, E., and Walther, J. B., *Arch. ges. Physiol.*, **265**, 355 (1957)
83. Donhoffer, S., Mestyán, G., Nagy, L., and Szegvári, G., *Acta Neuroveget. (Vienna)*, **16**, 390 (1957)
84. Donhoffer, S., Szegvári, G., Varga-Nagy, I., and Járαι, I., *Arch. ges. Physiol.*, **265**, 97 (1957)
85. Donhoffer, S., Szegvári, G., Varga-Nagy, I., and Járαι, I., *Arch. ges. Physiol.*, **265**, 104 (1957)
86. Dripps, R. D., Ed., *Natl. Acad. Sci.-Natl. Research Council, Publ. 451* (Washington, D. C., 447 pp., 1956)
87. Dugal, L. P., and Fortier, G., *Can. J. Biochem. and Physiol.*, **35**, 169 (1957)
88. Edholm, O. G., Fox, R. H., and Macpherson, R. K., *J. Physiol. (London)*, **134**, 612 (1956)
89. Edholm, O. G., Fox, R. H., and Macpherson, R. K., *J. Physiol. (London)*, **139**, 455 (1957)
90. Egdahl, R. H., and Richards, J. B., *Am. J. Physiol.*, **185**, 239 (1956)
91. Eisentraut, M., *Der Winterschlaf mit seinen ökologischen und physiologischen Begleiterscheinungen* (Gustav Fischer, Jena, Germany, 160 pp., 1956)
92. Elsner, R. W., *Alaskan Air Command, Arctic Aeromed. Lab., Ladd Air Force Base, Proj. No. 8-7951, Report No. 1* (1955)
93. Enger, P. S., *Acta Physiol. Scand.*, **40**, 162 (1957)
94. Erikson, H., Krog, J., Lange Andersen, K., and Scholander, P. F., *Acta Physiol. Scand.*, **37**, 35 (1956)
95. von Euler, C., and Söderberg, U., *Electroencephal. and Clin. Neurophysiol.*, **9**, 391 (1957)
96. von Euler, C., and Söderberg, U., *Acta Physiol. Scand.*, **42**, 112 (1958)
97. Fedor, E. J., Levine, M., Russ, C., and Fisher, B., *Surg. Forum*, **6**, 141 (1956)
98. Ferguson, I. D., and Levinson, N., *J. Physiol. (London)*, **128**, 608 (1955)
99. Ferrer, M. I., Ed., *Cold Injury. Trans. of the Fourth Conf.* (Josiah Macy, Jr., Foundation, New York, N. Y., 371 pp., 1956)
100. Fisher, E. R., Fedor, E. J., and Fisher, B., *Am. J. Physiol.*, **188**, 470 (1957)
101. Fisher, E. R., Fedor, E. J., and Fisher, B., *Arch. Surg.*, **75**, 817 (1957)
102. Fisher, B., Fedor, E. J., Lee, S. H., Weitzel, W. K., Selker, R., and Russ, C., *Surgery*, **40**, 862 (1956)
103. Fisher, B., Russ, C., and Fedor, E. J., *Am. J. Physiol.*, **188**, 473 (1957)
104. Forster, R. E., 2nd, and Ferguson, T. B., *Federation Proc.*, **10**, 44 (1951)
105. Fox, R. H., and Hilton, S. M., *J. Physiol. (London)*, **133**, 68P (1956)
106. Fox, R. H., and Hilton, S. M., *J. Physiol. (London)*, **137**, 43P (1957)
107. v. Frey, M., *Ber. süchs. Ges. (Akad.) Wiss.*, **47**, 166 (1895)

108. Froese, G., and Burton, A. C., *J. Appl. Physiol.*, **10**, 235 (1957)
109. Fuhrman, F. A., *J. Appl. Physiol.*, **10**, 139 (1957)
110. Fuhrman, F. A., *J. Appl. Physiol.*, **10**, 224 (1957)
111. Fuhrman, F. A., and Fuhrman, G. J., *Medicine*, **36**, 465 (1957)
112. Fuhrman, F. A., and Fuhrman, G. J., *J. Appl. Physiol.*, **11**, 45 (1957)
113. Garb, S., and Penna, M., *J. Appl. Physiol.*, **9**, 431 (1956)
114. Gaskell, P., *J. Physiol. (London)*, **131**, 647 (1956)
115. Gelineo, S., *Bull. acad. Serbe sci.*, **18**, 97 (1957)
116. Giaja, J., and Radulović, J., *Compt. rend.* **242**, 2039 (1956)
117. Giaja, J., and Radulović, J., *Nature*, **178**, 1286 (1956)
118. Giaja, J., Radulović, J., and Gavrilović, Z., *Compt. rend.* **244**, 27 (1957)
119. Giaja, J., and Rajevski, V., *Compt. rend. soc. biol.*, **149**, 449 (1955)
120. Giustina, G., and Meschia, G., *Arch. fisiol.*, **56**, 173 (1956)
121. Glaser, E. M., and Newling, P. S. B., *J. Physiol. (London)*, **137**, 1 (1957)
122. Glaser, E. M., and Whittow, G. C., *J. Physiol. (London)*, **136**, 98 (1957)
- 122a. Goldzweig, S. A., and Smith, A. U., *J. Physiol. (London)*, **132**, 406 (1956)
123. Goldzweig, S. A., and Smith, A. U., *J. Endocrinol.*, **14**, 40 (1956)
124. Golenhofen, K., *Arch. ges. Physiol.*, **266**, 665 (1958)
125. Gollan, F., and Nelson, I. A., *Proc. Soc. Exptl. Biol. Med.*, **95**, 485 (1957)
126. Gollan, F., Rudolph, G. G., and Olsen, N. S., *Am. J. Physiol.*, **189**, 277 (1957)
127. Good, A. L., and Sellers, A. F., *Am. J. Physiol.*, **188**, 447 (1957)
128. Good, A. L., and Sellers, A. F., *Am. J. Physiol.*, **188**, 451 (1957)
129. Graf, W., and Graf, K., *Acta Physiol. Scand.*, **41**, 140 (1957)
130. Grant, W. L., *S. African Mech. Eng.*, **6**, 109 (1956)
131. Grayson, J., and Mendel, D., *J. Physiol. (London)*, **133**, 334 (1956)
132. Haddy, F. J., Fleishman, M., and Scott, J. B., Jr., *Circulation Research*, **5**, 58 (1957)
133. Hahn, P., *Physiol. Bohemosloven.*, **5**, 291 (1956)
134. Hahn, P., Křeček, J., and Křečková, J., *Physiol. Bohemosloven.*, **5**, 283 (1956)
135. Hale, F. C., Westland, R. A., and Taylor, C. L., *J. Appl. Physiol.*, **12**, 20 (1958)
136. Hale, H. B., Sayers, G., Sydnor, K. L., Sweat, M. L., and Van Fossan, D. D., *J. Clin. Invest.*, **36**, 1642 (1957)
137. Hall, J. F., Jr., and Polte, J. W., *J. Appl. Physiol.*, **8**, 539 (1955/56)
138. Hammel, H. T., *Am. J. Physiol.*, **182**, 369 (1955)
139. Hammel, H. T., *J. Mammal.*, **37**, 375 (1956)
140. Hannon, J. P., *J. Appl. Physiol.*, **12**, 211 (1958)
141. Hannon, J. P., *Proc. Soc. Exptl. Biol. Med.*, **97**, 368 (1958)
142. Hannon, J. P., *Am. J. Physiol.*, **192**, 253 (1958)
143. Hannon, J. P., and Covino, B. G., *Am. J. Physiol.*, **192**, 121 (1958)
144. Hansen, A. T., Haxholdt, B. F., Husfeldt, E., Lassen, N. A., Munck, O., Sørensen, H. R., and Winkler, K., *Scand. J. Clin. & Lab. Invest.*, **8**, 182 (1956)
145. Hardenbergh, E., and Bamberg, P. G., *Am. J. Physiol.*, **188**, 461 (1957)
146. Hardy, J. D., Hammel, H. T., and Murgatroyd, D., *J. Appl. Physiol.*, **9**, 257 (1956)
147. Hardy, J. D., Stoll, A. M., Cunningham, D., Benson, W. M., and Greene, L., *Am. J. Physiol.*, **189**, 1 (1957)
148. Hart, J. S., *Rev. can. biol.*, **16**, 133 (1957)
149. Hellmann, K., Collins, K. J., Gray, C. H., Jones, R. M., Lunnon, J. B., and Weiner, J. S., *J. Endocrinol.*, **14**, 209 (1956)
150. Hellon, R. F., and Lind, A. R., *J. Physiol. (London)*, **133**, 132 (1956)

151. Hellon, R. F., and Lind, A. R., *J. Physiol. (London)*, **141**, 262 (1958)
152. Hemingway, A., and Birzis, L., *J. Appl. Physiol.*, **8**, 577 (1955/56)
153. Hendler, E., Crosbie, R., and Hardy, J. D., *J. Appl. Physiol.*, **12**, 177 (1958)
154. Henneman, D. H., Bunker, J. P., and Brewster, W. R., Jr., *J. Appl. Physiol.*, **12**, 164 (1958)
155. Hensel, H., *Ergeb. Physiol., biol. Chem. u. exp. Pharmacol.*, **47**, 166 (1952)
156. Hensel, H., *Arch. ges. Physiol.*, **257**, 371 (1953)
157. Hensel, H., *Z. vergleich. Physiol.*, **37**, 509 (1955)
158. Hensel, H., *Arch. ges. Physiol.*, **263**, 48 (1956)
159. Hensel, H., *Arch. ges. Physiol.*, **264**, 228 (1957)
160. Hensel, H., and Krüger, F. J., *Arch. ges. Physiol.*, **268**, 72 (1958)
161. Hensel, H., and Zotterman, Y., *J. Neurophysiol.*, **14**, 377 (1951)
162. Hertzman, A. B., *J. Appl. Physiol.*, **10**, 242 (1957)
163. Hiestand, W. A., Stemler, F. W., and Jasper, R. L., *Proc. Soc. Exptl. Biol. Med.*, **88**, 94 (1955)
164. Hillermann, J. P., and Wilson, W. O., *Am. J. Physiol.*, **180**, 591 (1955)
165. Holub, A., Forman, Z., and Ježková, D., *Nature*, **180**, 858 (1957)
166. Hong, S. K., *Am. J. Physiol.*, **188**, 137 (1957)
167. Horvath, S. M., Radcliffe, C. E., Hutt, B. K., and Spurr, G. B., *J. Appl. Physiol.*, **8**, 145 (1955/56)
168. Horvath, S. M., Spurr, G. B., Hutt, B. K., and Hamilton, L. H., *J. Appl. Physiol.*, **8**, 595 (1955/56)
169. Hsieh, A. C. L., and Carlson, L. D., *Am. J. Physiol.*, **190**, 243 (1957)
170. Hsieh, A. C. L., Carlson, L. D., and Gray, G., *Am. J. Physiol.*, **190**, 247 (1957)
171. Hügin, F., and Verzář, F., *Gerontologia*, **1**, 91 (1957)
172. Iampietro, P. F., Bass, D. E., and Buskirk, E. R., *J. Appl. Physiol.*, **10**, 398 (1957)
173. Iampietro, P. F., Buskirk, E. R., Bass, D. E., and Welch, B. E., *J. Appl. Physiol.*, **11**, 349 (1957)
- 173a. Iggo, A., *J. Physiol. (London)*, **143**, 47P (1958)
174. Imig, C. J., Roberson, W. J., and Hines, H. M., *Am. J. Physiol.*, **186**, 35 (1956)
175. Irving, L., and Hart, J. S., *Can. J. Zool.*, **35**, 497 (1957)
176. Irving, L., Schmidt-Nielsen, K., and Abrahamsen, N. S. B., *Physiol. Zool.*, **30**, 93 (1957)
177. Itoh, S., *Nagoya J. Med. Sci.*, **19**, 239 (1957)
178. Itzhaki, S., and Wertheimer, E., *Circulation Research*, **5**, 451 (1957)
179. Jacquez, J. A., Huss, J., McKeehan, W., Dimitroff, J. M., and Kuppenheim, H. F., *J. Appl. Physiol.*, **8**, 297 (1955/56)
180. Jacquez, J. A., Kuppenheim, H. F., Dimitroff, J. M., McKeehan, W., and Huss, J., *J. Appl. Physiol.*, **8**, 212 (1955/56)
181. Kandrór, I. S., and Rapoport, K. A., *Fiziol. Zhur. S.S.S.R.*, **43**, 60 (1957)
182. Kanter, G. S., *Am. J. Physiol.*, **188**, 443 (1957)
183. Kanter, G. S., and Lubinski, R. H., *Am. J. Physiol.*, **180**, 559 (1955)
184. Kantner, M., *Acta Neuroveget. (Vienna)*, **15**, 223 (1957)
185. Kanwisher, J. W., *Biol. Bull.*, **109**, 56 (1955)
186. Kanwisher, J., and Leivestad, H., *Norweg. Whaling Gazette*, **1**, 1 (1957)
187. Kassenaar, A. A. H., Lameyer, L. D. F., and Querido, A., *Acta Endocrinol.*, **21**, 37 (1956)
188. Kaufmann, W., Thauer, R., and Zöllner, G., *Arch. ges. Physiol.*, **261**, 189 (1955)
189. Kayser, C., *Rev. can. biol.*, **16**, 303 (1957)

190. Kayser, C., *Arch. sci. physiol.*, **11**, 7 (1957)
191. Kayser, C., Coraboeuf, E., and Gargouil, Y., *Compt. rend. soc. biol.*, **150**, 1789 (1957)
192. Keatinge, W. R., *J. Physiol. (London)*, **139**, 497 (1957)
193. Kerslake, D. McK., *J. Physiol. (London)*, **127**, 280 (1955)
194. Kreider, M. B., and Buskirk, E. R., *J. Appl. Physiol.*, **11**, 339 (1957)
- 194a. Kuhn, W., *Helv. Chim. Acta*, **39**, 1071 (1956)
195. Kundt, H. W., Brück, K., and Hensel, H., *Arch. ges. Physiol.*, **264**, 97 (1957)
196. Kundt, H. W., Brück, K., and Hensel, H., *Naturwissenschaften*, **44**, 496 (1957)
197. Kuno, Y., *Human Perspiration*, (Charles C Thomas, Publisher, Springfield, Ill., 416 pp., 1956)
198. Kyle, R. H., and Kirby, C. K., *Arch. Surg.*, **74**, 136 (1957)
199. Ladell, W. S. S., *J. Physiol. (London)*, **127**, 11 (1955)
200. L'Allemand, H., Brendel, W., and Usinger, W., *Anaesthesist*, **4**, 36 (1955)
201. Landgren, S., *Acta Physiol. Scand.*, **40**, 202 (1957)
202. Landgren, S., *Acta Physiol. Scand.*, **40**, 210 (1957)
203. LeBlanc, J. A., *J. Appl. Physiol.*, **9**, 395 (1956)
204. LeBlanc, J. A., *J. Appl. Physiol.*, **10**, 281, (1957)
205. LeBlanc, J. A., and Rosenberg, F. J., *J. Appl. Physiol.*, **11**, 344 (1957)
206. LeBlanc, J. A., and Rosenberg, F. J., *Proc. Soc. Exptl. Biol. Med.*, **96**, 234 (1957)
207. Lee, D. H. K., *King Ranch Centennial Conference: Breeding beef cattle for unfavorable environments*, 21 (University of Texas Press, Austin, Tex., 1955)
208. Lee, D. H. K., *Europ. Assoc. Animal Production, Publ. No. 5, Gen. Rept. No. 1*, 7 (1957)
209. Lee, D. H. K., *Climate and Economic Development in the Tropics* (Harper & Brothers, New York, N. Y., 1957)
210. Leidig, R., and Gray, G. M., *Am. J. Physiol.*, **188**, 507 (1957)
211. Lele, P. P., *J. Physiol. (London)*, **126**, 191 (1954)
212. Lele, P. P., Weddell, G., and Williams, C. M., *J. Physiol. (London)*, **126**, 206 (1954)
213. Levitt, J., in *Protoplasmalogia, Handbuch der Protoplasmaforschung*, **8**, Part 6 (Springer-Verlag, Vienna, Austria, 100 pp., 1958)
214. Lichton, I. J., *J. Appl. Physiol.*, **11**, 422 (1957)
215. Lim, P. K., and Grodins, F. S., *Am. J. Physiol.*, **180**, 445 (1955)
216. Lind, A. R., and Hellon, R. F., *J. Appl. Physiol.*, **11**, 35 (1957)
217. Lind, A. R., Hellon, R. F., Weiner, J. S., and Jones, R. M., *Brit. J. Ind. Med.*, **12**, 296 (1955)
218. Lovelock, J. E., and Smith, A. U., *Proc. Roy. Soc. (London)*, **145**, 427 (1956)
219. Lyburn, St. John E. F., *J. Physiol. (London)*, **134**, 207 (1956)
220. Lynch, H. F., and Adolph, E. F., *J. Appl. Physiol.*, **11**, 192 (1957)
221. MacFarlane, W. V., *Med. J. Australia*, **43**, Vol. II, 139 (1956)
222. Malmjeac, J., Plane, P., and Bogaert, E., *Compt. rend.*, **242**, 2764 (1956)
223. Marshall, J. M., *Circulation Research*, **5**, 664 (1957)
224. Marshall, J. M., and Williams, E. M. V., *J. Physiol. (London)*, **131**, 186 (1956)
225. Maruhashi, J., Miziguchi, K., and Tasaki, I., *J. Physiol. (London)*, **117**, 129 (1952)
226. Masoro, E. J., Asuncion, C. L., Brown, R. K., and Rapport, D., *Am. J. Physiol.*, **190**, 177 (1957)
227. Masoro, E. J., Felts, J. M., and Panagos, S. S., *Am. J. Physiol.*, **189**, 479 (1957)

228. Masoro, E. J., Panagos, S. S., Cohen, A. I., and Rapport, D., *Am. J. Physiol.*, **186**, 24 (1956)
229. Masterton, J. P., and Lewis, H. E., *Brit. J. Nutrition*, **2**, 346 (1957)
230. Matiushkina, N. A., *Fiziol. Zhur. S.S.S.R.*, **42**, 939 (1956)
231. Matsuda, K., Hoshi, T., and Kameyama, S., *Tôhoku J. Exptl. Med.*, **63**, 318 (1956)
232. Mavor, G. E., Harder, R. A., McEvoy, R. K., McCoord, A. B., and Mahoney, E. B., *Am. J. Physiol.*, **185**, 515 (1956)
233. McCance, R. A., Ungley, C. C., Crosfill, J. W. L., and Widdowson, E. M., *Med. Research Council (Brit.)*, *Spec. Rept. Ser.*, **291**, 1 (1956)
234. McMurrey, J. D., Bernhard, W. F., Taren, J. A., and Bering, E. A., Jr., *Surg., Gynecol. Obstet.*, **102**, 75 (1956)
235. Meehan, J. P., *Alaskan Air Command, Arctic Aerom. Lab., Ladd Air Force Base, Proj. No. 7-7951, Rept. No. 2* (1955)
236. Meryman, H. T., *Science*, **124**, 515 (1956)
237. Meryman, H. T., *Physiol. Revs.*, **37**, 233 (1957)
238. Miller, A. T., Jr., and Blyth, C. S., *J. Appl. Physiol.*, **12**, 17 (1958)
239. Mills, A. W., *J. Appl. Physiol.*, **9**, 447 (1956)
240. Morales, P., Carbery, W., Morello, A., and Morales, G., *Ann. Surgery*, **145**, 488 (1957)
241. Müller, E. A., and Wenzel, H.-G., *Intern. Z. angew. Physiol.*, **16**, 373 (1957)
242. Murray, R. W., *Nature*, **176**, 698 (1955)
243. Murray, R. W., *J. Exptl. Biol.*, **33**, 798 (1956)
244. Murray, R. W., *Nature*, **179**, 106 (1957)
245. Niazi, S. A., and Lewis, F. J., *J. Appl. Physiol.*, **10**, 137 (1957)
246. Nicholls, D., and Rossiter, R. J., *Am. J. Physiol.*, **187**, 11 (1956)
247. Nicolai, L., *Arch. ges. Physiol.*, **263**, 453 (1956/57)
248. Nicolai, L., *Arch. ges. Physiol.*, **266**, 308 (1958)
249. Osiba, S., *Japan. J. Physiol.*, **7**, 355 (1957)
250. Otis, A. B., and Jude, J., *Am. J. Physiol.*, **188**, 355 (1957)
251. Percy, M., Robinson, S., Miller, D. I., Thomas, J. T., Jr., and DeBrotta, J., *J. Appl. Physiol.*, **8**, 621 (1955/56)
252. Petrunj, N. M., *Doklady Akad. Nauk S.S.S.R.*, **111**, 228 (1956)
253. Pichotka, J., Höfler, W., and Reissner, J., *Arch. exptl. Pathol. u. Pharmacol., Naunyn-Schmiedeberg's*, **223**, 217 (1954)
254. Precht, H., Christophersen, J., and Hensel, H., *Temperatur und Leben* (Springer-Verlag, Berlin, Göttingen, Heidelberg, Germany, 514 pp., 1955)
255. Ralli, E. P., Kuhl, W. J., Jr., Gershberg, H., Beck, E. M., Street, E. R., and Laken, B., *Metabolism, Clin. and Exptl.*, **5**, 170 (1956)
256. Randall, W. C., Cox, J. W., Alexander, W. F., Coldwater, K. B., and Hertzman, A. B., *J. Appl. Physiol.*, **7**, 688 (1954/55)
257. Raths, P., *Z. Biol.*, **110**, 62 (1958)
258. Redisch, W., Tangco, F. T., Wertheimer, L., Lewis, A. J., and Steele, J. M., *Circulation*, **15**, 518 (1957)
259. Keissmann, K. R., and Kapoor, S., *Am. J. Physiol.*, **184**, 162 (1956)
260. Rennie, D. W., and Adams, T., *J. Appl. Physiol.*, **11**, 201 (1957)
261. Riberi, A., Siderys, H., and Shumacker, H. B., Jr., *Ann. Surg.*, **143**, 216 (1956)
262. Richards, J. B., and Egdahl, R. H., *Am. J. Physiol.*, **186**, 435 (1956)
263. Riley, P. A., Jr., Mixon, B. M., Jr., and Barila, T. G., *Surgery*, **42**, 936 (1957)

264. Robinson, K. W., Howard, B., and MacFarlane, W. V., *Med. J. Australia*, **42**, 11, 756 (1955)
265. Robinson, K. W., and MacFarlane, W. V., *Australian J. Biol. Sci.*, **9**, 130 (1956)
266. Robinson, K. W., and MacFarlane, W. V., *J. Appl. Physiol.*, **12**, 13 (1958)
267. Robinson, S., Maletich, R. T., Robinson, W. S., Rohrer, B. B., and Kunz, A. L., *J. Appl. Physiol.*, **8**, 615 (1955/56)
268. Rodahl, K., *Alaskan Air Command, Arctic Aeromed. Lab., Ladd Air Force Base, Tech. Rept. 57-21* (1957)
269. Rodahl, K., and Bang, G., *Alaskan Air Command, Arctic Aeromed. Lab., Ladd Air Force Base, Tech. Rept. 57-36* (1957)
270. Rodahl, K., and Rennie, D. W., *Alaskan Air Command, Arctic Aeromed. Lab., Ladd Air Force Base, Proj. 8-7951, Rept. 7* (1957)
271. Roddie, I. C., and Shepherd, J. T., *Clin. Sci.*, **15**, 433 (1956)
272. Roddie, I. C., and Shepherd, J. T., *J. Physiol. (London)*, **131**, 657 (1956)
273. Roddie, I. C., Shepherd, J. T., and Whelan, R. F., *J. Physiol. (London)*, **134**, 444 (1956)
274. Roddie, I. C., Shepherd, J. T., and Whelan, R. F., *J. Physiol. (London)*, **136**, 489 (1957)
275. Roddie, I. C., Shepherd, J. T., and Whelan, R. F., *J. Physiol. (London)*, **138**, 445 (1957)
276. Roddie, I. C., Shepherd, J. T., and Whelan, R. F., *Clin. Sci.*, **16**, 67 (1957)
277. Sarajas, H. S. S., *Am. Heart J.*, **51**, 298 (1956)
278. Sarajas, H. S. S., Nyholm, P., and Suomalainen, P., *Nature*, **181**, 612 (1958)
279. Schmidt-Nielsen, B., Schmidt-Nielsen, K., Houpt, T. R., and Jarnum, S. A., *Am. J. Physiol.*, **185**, 185 (1956)
280. Scholander, P. F., van Dam, L., Kanwisher, J. W., Hammel, H. T., and Gordon, M. S., *J. Cellular Comp. Physiol.*, **49**, 5 (1957)
- 280a. Scholander, P. F., Hammel, H. T., Hart, J. S., LeMesurier, D. H., and Steen, J., *J. Appl. Physiol.*, **13**, 211 (1958)
281. Scholander, P. F., Hammel, H. T., Lange Andersen, K., and Løynning, Y., *J. Appl. Physiol.*, **12**, 1 (1958)
282. Scholander, P. F., and Krog, J., *J. Appl. Physiol.*, **10**, 405 (1957)
283. Scholander, P. F., Lange Andersen, K., Krog, J., Vogt Lorentzen, F., and Steen, J., *J. Appl. Physiol.*, **10**, 231 (1957)
284. Schreider, E., *Nature*, **179**, 915 (1957)
285. Schwartz, I. L., and Thaysen, J. H., *J. Clin. Invest.*, **35**, 114 (1956)
286. Segar, W. E., Riley, P. A., Jr., and Barila, T. G., *Am. J. Physiol.*, **185**, 528 (1956)
287. Sellers, E. A., and You, R. W., *Brit. Med. J.*, **1**, 815 (1956)
288. Severinghaus, J. W., Stupfel, A., and Bradley, A. F., *J. Appl. Physiol.*, **10**, 349 (1957)
289. Shumacker, H. B., Jr., Riberi, A., Boone, R. D., and Kajikuri, H., *Ann. Surg.*, **143**, 223 (1956)
290. Sinclair, D. C., *Brain*, **78**, 584 (1955)
291. Smirnov, K. M., and Skliartchik, E. L., *Fiziol. Zhur. S.S.S.R.*, **43**, 389 (1957)
292. Smith, A. U., *Proc. Roy. Soc. (London)*, **145**, 407 (1956)
- 292a. Smith, A. U., *Biol. Revs. Cambridge Phil. Soc.*, **33**, 197 (1958)
293. Snell, E. S., *J. Physiol. (London)*, **125**, 361 (1954)
294. Söderberg, U., *Experientia*, **12**, 299 (1956)
295. Spurr, G. B., Horvath, S. M., Hamilton, L. H., and Hutt, B. K., *Am. J. Physiol.*, **186**, 47 (1956)

296. Spurr, G. B., Hutt, B. K., and Horvath, S. M., *Am. Heart J.*, **50**, 551 (1955)
297. Spurr, G. B., Hutt, B. K., and Horvath, S. M., *J. Appl. Physiol.*, **11**, 58 (1957)
298. Steen, J., *Acta Physiol. Scand.*, **39**, 22 (1957)
299. Steen, J., and Enger, P. S., *Am. J. Physiol.*, **191**, 157 (1957)
300. Stillwell, G. K., Hemingway, A., and Kottke, F. J., *J. Appl. Physiol.*, **8**, 223 (1955/56)
301. Ström, G., *Acta Physiol. Scand.*, **20**, Suppl. 70, 47 (1950)
302. Ström, G., *Acta Physiol. Scand.*, **21**, 271 (1950)
303. Stupfel, M., and Severinghaus, J. W., *J. Appl. Physiol.*, **9**, 380 (1956)
304. Suda, I., Koizumi, K., and Brooks, C. McC., *Am. J. Physiol.*, **189**, 373 (1957)
305. Sullivan, B. J., and Flynn, P. M., *Proc. Soc. Exptl. Biol. Med.*, **94**, 346 (1957)
306. Sullivan, B. J., and LeBlanc, M. F., *Am. J. Physiol.*, **189**, 501 (1957)
307. Sullivan, B. J., and Towle, L. B., *Am. J. Physiol.*, **189**, 498 (1957)
308. Sutherland, G. B., and Campbell, D. H., *Proc. Soc. Exptl. Biol. Med.*, **91**, 64 (1956)
309. Swanson, H. E., *Endocrinology*, **60**, 205 (1957)
310. Teichner, W. H., *J. Appl. Physiol.*, **11**, 333 (1957)
311. Teichner, W. H., and Kobrick, J. L., *J. Exptl. Psychol.*, **49**, 122 (1955)
312. Thron, H. L., *Arch. ges. Physiol.*, **263**, 127 (1956/57)
313. Thron, H. L., Scheppokat, K. D., Heyden, A., and Gauer, O. H., *Arch. ges. Physiol.*, **266**, 150 (1958)
314. Ueno, T., *Nagoya J. Med. Sci.*, **20**, 75 (1957)
315. Usinger, W., *Arch. ges. Physiol.*, **265**, 365 (1957)
316. Vendrik, A. J. H., and Vos, J. J., *J. Appl. Physiol.*, **11**, 211 (1957)
317. Vere, D. W., *J. Physiol. (London)*, **140**, 350 (1958)
318. Wallace, J. M., and Stead, E. A., *Circulation Research*, **5**, 650 (1957)
319. Webb, P., *Alaskan Air Command, Arctic Aeromed. Lab., Ladd Air Force Base, Proj. No. 7-7951, Rept. No. 3* (1955)
320. Webb, P., Garlington, L. N., and Schwarz, M. J., *J. Appl. Physiol.*, **11**, 41 (1957)
321. Welch, B. E., Levy, L. M., Consolazio, C. F., Buskirk, E. R., and Dee, T. E., *U. S. Army Med. Nutrition Lab. Rept. No. 202* (Denver, Colo., 24 pp., 1957)
322. Welch, B. E., Marcinek, J. G., Mann, J. B., Grotheer, M. P., Friedemann, T. E., Iampietro, P. F., Vaughn, J. A., and MacLeod, A., *U. S. Army Med. Nutrition Lab. Rept. No. 190* (Denver, Colo., 22 pp., 1956)
323. Wenzel, H.-G., and Müller, E. A., *Intern. Z. Angew. Physiol.*, **16**, 335 (1957)
324. Werner, A. Y., Dawson, D., and Hardenbergh, E., *Science*, **124**, 1145 (1956)
325. Whitney, R. J., *J. Physiol. (London)*, **125**, 1 (1954)
326. Whittow, G. C., *Nature*, **176**, 511 (1955)
327. Wilber, C. G., *Am. J. Physiol.*, **190**, 457 (1957)
328. Wilber, C. G., *Human Biol.*, **29**, 329 (1957)
329. Wilber, C. G., and Robinson, P. F., *J. Appl. Physiol.*, **12**, 214 (1958)
330. Witt, I., *Arch. ges. Physiol.*, **268**, 39 (1958)
331. Woodhall, B., Reynolds, D. H., *Proc. Soc. Exptl. Biol. Med.*, **97**, 194 (1958)
332. Wyndham, C. H., and Guttman, L., *S. African J. Med. Sci.*, **20**, 93 (1955)
333. Wyndham, C. H., and Jacobs, G. E., *J. Appl. Physiol.*, **11**, 197 (1957)
334. Wyndham, C. H., and Morrison, J. F., *Nature*, **178**, 869 (1956)
335. Wynn, V., *Clin. Sci.*, **15**, 297 (1956)
336. Young, D. R., and Cook, S. F., *Am. J. Physiol.*, **181**, 72 (1955)
337. Žerebčenko, P. G., *Fiziol. Zhurn. Akad. Nauk Ukr. R.S.R.*, **2**, 21 (1956)

KIDNEY, WATER AND ELECTROLYTE METABOLISM^{1,2}

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INTRODUCTION

A number of pertinent reviews on the kidney and its role in water and electrolyte metabolism have appeared during the span of this review. That by Darrow & Hellerstein (1) on the interpretation of certain changes in body water and electrolytes is of particular interest to the renal physiologist. Schmidt-Nielsen has written on urea excretion in mammals (2), offering the interesting proposal that the urea concentration gradients found in renal tissue may be based on the countercurrent multiplier principle. An excellent review of the physiology of the mammalian antidiuretic hormone by Thorn (3) has appeared. Also concerned with the neurohypophysis is the monograph edited by Heller (4). Related particularly to the present area of coverage are articles by Andersson (5) on polydipsia, antidiuresis, and milk ejection caused by hypothalamic stimulation, one by Brooks & Pickford (6) on conditions under which posterior pituitary hormones increase Na and K excretion by the kidney, by Wirz (7) on the location of antidiuretic action in the mammalian kidney, and by Sawyer (8) on the antidiuretic action of neurohypophysial hormone in amphibia. Smith has written on salt and water volume receptors (9), and Wesson (10) on glomerular and tubular factors in the renal excretion of sodium chloride.

At the time of completion of the reading for this review an extensive symposium of renal structure and function appeared with articles on the electron microscopy of the kidney by Rhodin (11), on the technique of renal biopsies by Brun & Raaschou (12), on the structure, perfusion, and function of nephrons by Bradley & Wheeler (13), on the excretion of weak acids and bases by Milne *et al.* (14), and on dilution and concentration of the urine and ADH action by Berliner *et al.* (15). Pitts (16) discussed the mechanism of action of diuretics, and Relman & Schwartz considered (17) kidney function under conditions of K depletion. Taggart (18) brought up to date the thinking on the mechanisms of tubular transport, and Mudge (19) concluded the series with a discussion of clinical patterns of tubular dysfunction. The

¹ The survey of literature pertaining to this review was concluded June 1, 1958.

² The following abbreviations are among those used in this chapter: PAH (para-aminohippuric acid); GFR (glomerular filtration rate); ADH (antidiuretic hormone); RBF (renal blood flow); 2,4-DNP (2,4-dinitrophenol); Tm (tubular maximum).

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timely coverage of these areas in this symposium adds an excellent supplement to the present review.

ANATOMICAL ASPECTS

The interesting zone concerned with ultrafiltration in the glomerulus has been closely scrutinized with electron microscopy. Rhodin (11) holds for perforations in the attenuated cytoplasm of the endothelial layer of rats and mice (500 Å thick) of a size 500 to 1000 Å, despite the contention of Yamada (20) that these perforations are closed by a thin membrane. The fenestrations have been observed by Hall (21), and occur in the endothelial lining of the amphibian glomerulus (22) as well as in the marsupial (23) and the avian glomerulus (24). The size of these is such that they would offer restraint only to passage of blood cells, and not to plasma. The basement membrane (lamina densa) of the glomerulus is described by Rhodin as being a structureless layer 800 Å thick, composed of mucoproteins, through which ultrafiltration apparently proceeds without hindrance. Some workers appear to find this composed of three layers of different density (23). The latest work shows no pores in the lamina densa (25). The epithelial cells of Bowman's capsule branch into trabeculae and interdigitating pedicels. Hall (21) suggests that the "slit pores" formed by the interdigitating pedicels may be the limiting pore for filtration. The space between adjacent folds appears to be only 80 Å wide, and should restrict passage of plasma proteins. The length of the "slit pores" markedly increases the area available for filtration. Hall has described another mechanism which aids ultrafiltration and is concerned with the organization of the glomerular capillaries. This consists of "through" capillaries and smaller cross-anastomotic capillaries like rungs of a ladder between the direct communicating vessels. Pressure in these vessels should be maximal and flow minimal. Also, if plasma skimming occurs, they should be relatively cell-free, thus increasing the plasma volume within the capillaries.

The intricate details of tubular structure revealed by the electron microscope have been well described by Rhodin and need not be repeated here. In view of the special function currently assigned the loop of Henle and the collecting tubule, Rhodin's comments may be relevant, nevertheless. He states that the thin segment of Henle would, from structural appearance, function like ordinary capillary cells. Looking upon its role as one merely of osmosis, this tallies well with the relatively small number of mitochondria. On the other hand, he finds a multitude of cytoplasmic lamellae, implying that osmosis may be facilitated by the enlarged intercellular surface. In the collecting tubule, Rhodin observes two types of cell, the light and dark (intercalated), the latter a result of relatively abundant ribonucleic acid granules. The latter also contain more mitochondria. He believes the light cells may be concerned with the absorption of water, whereas the dark (intercalated) cells resemble those found in the distal convoluted tubule, so that they may participate in secretion of NH_3 and the acidification of the urine.

RENAL BLOOD FLOW

The mechanism of the autonomy of the renal circulation continues to receive attention. The hypothesis of Pappenheimer & Kinter (26, 27) has invoked plasma skimming and cell separation as an explanation of autoregulation. The effective viscosity of the blood increases as a function of the third power of the red cell concentration. Consequently, small changes in the degree of plasma skimming in the interlobular arteries will produce relatively large changes in the effective viscosity of the cell-rich component of blood reaching the outer arterioles of the cortex. The degree of skimming would be a function of the arterial pressure. Thus, with an increase in pressure a fluid of greater viscosity is formed with little change in flow.

Nevertheless, Waugh (28) found that autonomy persisted in excised, denervated dog kidneys in the presence of perfusion of blood with hematocrit in the range 9 to 20 per cent, and with oxygenated colloidal solutions (6 per cent dextran and 4 per cent polyvinylpyrrolidone), all in the range of *ca.* 80 to 170 mm. Hg arterial pressure. It was not abolished by anoxia of 18 to 23 min., but when procaine was infused the autoregulation was eliminated. He suggests as the mechanism a myogenic response of the afferent arterioles, with vasoconstriction in response to a rise in pressure, and vasodilatation in response to lowering of pressure. The action of procaine is described as a direct one on the smooth musculature of the arterioles. Since anoxia does not depress the autoregulation, the mechanism is presumably not a result of nerve activity, since nerve function should be abolished by relatively brief periods of anoxia.

Thompson *et al.* (29) also found that the hematocrit had no influence on the autoregulation; it persisted at hematocrits in the range 4 to 10 per cent and arterial pressures from *ca.* 50 to 170 mm. Hg. Nor was the concept of postglomerular separation of erythrocytes and plasma strongly supported, since the PAH extraction, which averaged 75 per cent in the control, was only reduced to 62 per cent in the anemic animals. Another concept of Pappenheimer & Kinter (26) was examined, namely whether the central glomeruli receive more plasma and produce more filtrate than those peripherally placed. Were this true, anemia would equalize the filtration rates and introduce a splay into the glucose titration curve of the dog. This was found not to be the case by Kessler *et al.* (30). Dogs with anemia (4 to 11 per cent hematocrit) showed no difference in the glucose titration curves from the normal.

On perfusion of fresh dog kidneys with kerosene and with mixtures of mineral oil (providing a non-Newtonian liquid with no anomalous viscosity, water immiscible, which should not create edema), a curvilinear pressure-flow relationship was found, convex to the pressure axis (31), suggesting passive expansion of vessels with increasing intraluminal pressure. It was determined that the reduction in resistance was largely upstream from the arcuate veins and did not result from operation of arteriovenous or transmedullary shunts. As the arterial pressure was lowered, flow stopped because of a small residual

intrarenal pressure (at about 2 mm. Hg.). This was, however, not taken to be "critical closure" in the sense employed by Burton. The lack of autonomy with mineral oil perfusion, even when oxygenated, could not be satisfactorily explained and seems contradictory to Waugh's earlier explanation of autonomy (28). When, in oil perfused kidneys, venous and arterial pressure were equally increased, flow and resistance remained constant, even though the kidney volume and "tenseness" visibly increased (32). Transmural pressure remained constant, as did the arteriovenous pressure difference which produces the flow. Some interesting added observations on the oil perfused, isolated kidney within a pressure chamber showed that an elevated extrarenal pressure caused the transmural venous pressure to fall, and the organ became flaccid, rather than engorged. Hence, such phenomena as increased abdominal pressure could not cause venous congestion of the kidney as some earlier investigations have averred.

The low hematocrit of the renal blood previously described by Pappenheimer & Kinter (26) and by others, has been attributed by Swann *et al.* (33) to a large "interstitial space" in the kidney, rather than preferential distribution of cells into red cell shunts and plasma into the peritubular capillary network. In the view of Swann *et al.*, the peritubular capillaries leak out plasma easily, keeping only red cells behind. Thus a specialized plasma circulation to the tubular portions of the nephron is furnished. Good-*yer et al.* (34) found the renal hematocrit extremely low (5 to 21 per cent at comparable arterial hematocrits of 36 to 52 per cent) in keeping with findings of Swann. These estimates were obtained with I^{131} for plasma volume, and cell volume was measured with the acid hematin method on homogenized dog kidneys. They too consider that the albumin space is larger than might reasonably be considered to be entirely within the direct renal circulation, and suggest two reasonable possibilities: that it might include cell-free and cell-poor capillaries, or lymphatic channels and interstitial spaces.

Simultaneous tagged red cell and tagged plasma albumin concentration-time curves were obtained from the renal vein without recirculation in dog kidneys by Lilienfield *et al.* (35). The mean circulation time of red cells averaged 6.4 sec., while that for plasma was 7.6 sec. The observation of an initially high ratio of red cells to plasma activity in the blood which first appeared in the renal vein led them to the inference that the red cells are preferentially shunted into those vascular pathways of shortest length; the results are not attributed to axial streaming. The circulating dynamic intrarenal hematocrit was determined and found to average 0.89 ± 0.03 of the large vessel hematocrit. Note that this compares to a ratio of 0.48 obtained by Pappenheimer & Kinter (26). These authors computed the intrarenal circulating blood volume to average 24 ml./100 gm. of kidney. Ochwaldt (36), using techniques similar to those of Lilienfield *et al.*, found a ratio of cell to plasma transit time of 0.86 compared to 0.83 of the latter. Likewise, the dynamic intrarenal hematocrit averaged 0.90 of the arterial hematocrit and was unaffected by potassium cyanide poisoning and lowered arterial pressure.

The reason for difficulty in maintaining normal blood flow through an externally perfused kidney has been investigated by Brull & Louis-Bar (37). When blood comes in contact with rubber tubing, glass, and plastic ware, even though siliconized or wax-coated, a vasoconstrictor substance is produced. They correlated this with a marked drop in the platelet count of the blood, and they speculated that serotonin release might be the basis for the vasoconstrictor action. Investigations by Hollander *et al.* (38) showed that serotonin reduced PAH and inulin clearances in man to a small but statistically significant degree. Along with this went decreases in urine volume, and Na and K excretion.

Although it is generally conceded that the barbiturates decrease renal blood flow [presumably by vasoconstrictor action (39)], no changes were noted during relatively brief periods of observation in dogs (40). Endotoxin from *E. coli* has a vasoconstrictor action on the kidney (41). The effects of hypothermia on renal function have been restudied (42). Marked decreases in GFR and RBF were produced by cooling to 23 to 24°C., the result of vasoconstriction produced by reduction of cardiac output. Effects on urine volume were variable, but Tm of glucose and PAH decreased.

Burch & Philips (43) have employed a large volume oncometer to study the volume pulse waves of the kidney in the dog. This unique application has permitted an analysis of inflow and outflow of blood during each cardiac cycle. The expected early rise in rate of inflow during the cycle was noted, with a lower rate of inflow at the terminal portion of the pulse cycle than at the beginning. The outflow curve showed an initial dip while the inflow curve was rising, then a slower rise to a delayed and lower peak than the inflow curve; both terminated with a lower rate of flow at the end of the cycle.

Differential thermocouples have been employed by Janssen & Grupp (44) to analyze flow and metabolism in the kidney. The temperature of the cortex is somewhat higher than that of the medulla, and it is least in the zone between. When the circulation is interrupted for 1 to 3 min., the temperature rises more rapidly in the cortex than in the medulla as heat accumulates in the metabolically more active zone. Hix has demonstrated an ureterorenal reflex in dogs; insertion of a catheter caused ipsilateral vasoconstriction (45). Action potentials of the renal nerves of cats under chloralose or chloralose-urethane anesthesia have been studied by Engelhorn during a variety of reflex mechanisms (46).

TUBULAR FUNCTION: METABOLISM AND CLEARANCE OF ORGANIC SUBSTANCES

Renal slice studies.—White has proposed two phases concerned with the renal transport of PAH based on studies done on rat, guinea pig, and rabbit renal slices (47): a slow phase from blood to tubule cell, and a rapid phase from cell to tubule lumen. The former is inhibited by 2,4-DNP, cyanide, and thiopental, while the latter is not. Presumably some back diffusion from cell to blood may occur, so that the ultimate exchange may be looked upon

as a flux. Thus, the slow phase is dependent on aerobic metabolism. The effects of sodium thiopental are elaborated on. It is apparently an inhibitor of coenzyme A; it depresses the uptake of PAH without altering Q_{O_2} , and completely inhibits the stimulatory effect of acetate on the uptake of PAH. Methylated xanthines caused a reduction in PAH uptake in rabbit kidney slices (48), with aminophylline being most effective. It is concluded that the most logical explanation for the effects noted was that the xanthines increased the cellular permeability, allowing more PAH to back-diffuse. Rabbit kidney slices efficiently concentrate 5-hydroxytryptamine, a substance produced in large amounts by malignant carcinoids (49). The uptake is depressed by anoxia and 2,4-DNP, and is enhanced by acetate. It is of interest that the metabolic end-product, 5-hydroxyindolacetic acid, had a renal clearance of ca. 350 ml./min. in a patient. Probenecid (Benemid) depressed its secretion, and an excretory mechanism similar to PAH was suggested.

Deyrup (50) has studied the factors influencing the uptake and loss of radiosulfate by rat renal cortical tissue. Phlorizin was observed to accelerate the accumulative process, while variable loss of S^{35} could be induced by anoxia, 2,4-DNP, mercuric ion, and the cations Na and Ca. Cohen, Berglund & Lotspeich (51) had earlier shown that phlorizin elevates the reabsorption of acetoacetate, PO_4 , and SO_4 , by removing the glucose competitive effect.

Secretory mechanisms.—Phlorizin is excreted by filtration and tubular secretion by the dog kidney (52). The ratio of its clearance to that of creatinine was depressed with elevation of plasma concentration, and both Benemid and PAH blocked its tubular secretion. The substance was excreted via the renal portal system of the chicken and by the aglomerular fish (*Lophius americanus*). During phlorizin administration, phlorizin glucuronide was found in the blood and urine, and was determined to be cleared at approximately the level of glomerular filtration. When Benemid was given, a marked tubular secretion of glucuronide was uncovered. The affinity of phlorizin for the secretory pathway apparently was higher than that of the glucuronide; when Benemid blocked the former, the pathway became available for the glucuronide, whose secretion was less Benemid-sensitive. Essentially similar findings were reported by Whittaker (53).

When epinephrine was infused into dogs, the excretion of β -catechol ethanolamines was greatly increased and was principally the free secondary amine, i.e., epinephrine (54). Its clearance was less than PAH but greater than GFR, indicating tubular secretion. Some metabolism of the substance must occur in the kidney, however, because the amount present in the urine is less than that which is extracted by the kidney. During hyperthyroidism, there is an increase in the percentage of infused epinephrine excreted in the urine (55). Since this was correlated with an increase in PAH clearance, it might be a reflection of increase in RBF.

Several studies have been concerned with the renal metabolism of histamine. The whole blood clearance of C^{14} -histamine in dogs was only 0.5 to 0.7 ml./min. (56), with extraction of 0.7 to 0.8, so that only a small part of

that extracted appeared in the urine. When aminoguanidine, an histaminase inhibitor, was given, the excretion rose, but some metabolic removal continued as the result of activity of other histaminases. When the labelled histamine was given by steady infusion into one renal artery, the loaded kidney excreted several times more C^{14} -labelled methylhistamine, methylimidazoleacetic acid, and imidazoleacetic acid than the opposite control kidney, indicating the inactivation of histamine by methylating enzymes (57). It can be added here that the canine kidney is able to convert histidine to histamine. When labelled histidine is given into the artery, labelled histamine appears in the urine (58).

The renal handling of histamine was also examined in the hen by Lindahl & Sperber (59), taking advantage of the portal system in this species to study a possible secretory system. They could demonstrate no Tm with infusion rates up to 667 $\mu\text{g}/\text{min}$. 2-Benzyl-2-imidazoline hydrochloride (Priscoline) consistently reduced secretion of histamine, with no effect on simultaneous phenol red secretion. Cyanine inhibited excretion of both histamine and phenol red. Lindahl and Sperber conclude that the secretory mechanism is one common for organic bases, and direct evidence for this is supplied by the work of Kandel *et al.* (60) who also demonstrated in the renal portal system of the hen competition between N' -methylnicotinamide (NMN) and Priscoline. Thus, NMN, tetraethylammonium (TEA), mepiperphenidol (Darstine), Priscoline, and histamine appear to be excreted by a common mechanism.

A 26.5 per cent reduction Tm_{PAH} resulted from the action of pentobarbital anesthesia in dogs (61). This was reversed by infusion of acetate. It was speculated that the barbiturate acts by uncoupling oxidative phosphorylation.

The extraction of citric acid by the dog was 1.37 to 1.57 times greater than that of creatinine, but less than that of PAH (62). The figure largely reflects renal utilization, since only small amounts are excreted into the urine.

Reabsorptive mechanisms.—The mechanism of clearance of barbiturates, based on the clearance analysis of phenobarbital by Waddell & Butler (63), is filtration of the unbound moiety and passive back diffusion. The clearance is elevated in alkaline urine, with a ratio to creatinine between 0.3 and 0.7, depending on the urine flow. Benemid does not affect its clearance. Similar to phenobarbital, the clearance of (5,5-dimethyl-2,4-oxazolinedione (DMO) is greater in alkaline urine, and also varies with the urine flow (64), suggesting passive back diffusion. The basic mechanism for the difference in rate of reabsorption in alkaline versus acid urine involves the consideration that the tubular epithelium is permeable to the lipide-soluble undissociated forms of acids and bases but impermeable to ionic forms. (DMO is an acid with a pK' of 6.13 at 37° and an ionic strength of 0.16.)

Reabsorption of lysine was compared in cystinurics and normals and found to have a clearance of 55 ml./min., with 45 per cent reabsorption, compared to more than 99 per cent reabsorption by normal subjects (65).

GFR was approximately normal in the cystinurics, averaging 105 ml./min. It is suggested that in cystinuria the transport system is present only in trace amounts. With loading of lysine no additional amounts are reabsorbed, in sharp contrast with the aminoaciduria of nephrosis, chronic renal disease, Wilson's disease, De Toni-Fanconi, and Lowe syndrome. In these, with increased load, both the amounts reabsorbed and amounts excreted are increased.

I^{131} clearance in rats exceeds that of Na and Cl (66) suggesting that the tubules are less permeable to I than to Na and Cl. The authors believe that although passive reabsorption of iodide may occur, an active transport system exists. Evidence is based on the marked increase in I^{131} clearance with increase in plasma concentration, suggesting saturation of a reabsorptive system. This is further suggested by the blockage of reabsorption that occurs when the sodium salts of chloride, perchlorate, bromide, and bicarbonate are given. Certainly Br and Cl might be competitors for loci on the iodide carrier.

The clearances of ribonuclease (67) and amylase (68) are much lower than simultaneous GFR, suggesting filtration and reabsorption; but details of mechanisms are not clear, since in the case of ribonuclease there may be plasma binding, and in the case of amylase the degree of loading appeared to be inadequate to elucidate the nature of the reabsorptive mechanism. Competition between the reabsorption of pyruvate and fructose was indicated by the work of Hoenig (69), and Huffman *et al.* (70) showed a significant interrelationship between phosphate clearance and the blood glucose level.

The manner of excretion of uric acid in normal and gouty human subjects has been extensively re-examined during the past year. The subject has been reviewed by Gutman (71). Current concepts are that urate is completely filterable and that 90 to 95 per cent of the load is reabsorbed, presumably in the proximal tubule. There is, however, some urate excretion to all loads. In a study of normal males the mean urinary excretion was 493 ± 159 μ g. per min., with a C_{urate} of 8.7 ± 2.5 ml./min. (72). Gouty males showed a mean excretion of 663μ g. $\pm 235 \mu$ g., 78 per cent falling within 2 S.D. of the normal mean. Urate clearance averaged 7.5 ± 2.4 ml./min., falling almost entirely within 2 S.D. of the normal values, suggesting no specific tubular defect. The higher excretion rate in gout is caused by increase in plasma urate concentration, the result of overproduction.

The excretion of uric acid has recently been proposed to include both a reabsorptive and a secretory mechanism (72), although the evidence for the latter is only suggestive. The hypothesis is based on the poor correlation between C_{IX} and urate clearance, and the unpredictability of urate excretion in relation to filtered urate load and the reabsorptive capacity of the tubules. The authors suggest as further evidence the fact that uric acid retention results from action of uricosuric drugs in low dosage (e.g., salicylate and phenylbutazone) resulting from inhibition of a secretory mechanism. The presumption is that the mechanism may be analogous to that postulated for K excretion.

Several drugs have been shown to decrease the excretion of uric acid (73, 74). Pyrazinamide elevated plasma levels of uric acid and reduced urinary excretion by one-third to one-half in patients with no change in C_{IN} , implying enhanced tubular reabsorption. Pyrazinoic acid acts similarly, although the possibility is not excluded that these drugs may decrease urinary excretion by blocking the hypothetical tubular secretion. Infusion of sodium lactate causes a marked reduction in C_{urate} (75), without any regular alteration in C_{IN} and C_{PAH} , implying mechanisms similar to the above.

Zoxazolamine is a new potent uricosuric agent (76). With a daily dosage of 500 to 750 mg., serum uric acid dropped from 8.6 to less than 4 mg. per cent and the uric acid/creatinine clearance ratio rose from 5 to 15 per cent. *p*-Aminosalicylic acid is somewhat uricosuric (73). Benemid reduces the hyperuricemic effect of pyrazinamide in some subjects (73) and will enhance urate clearance (75). The uricosuric action of salicylates is well known. Yu *et al.* (75) report a case in which C_{urate} increased from 11.8 to 51.2 ml./min. by injection of salicylate; it is interesting that this effect could be reversed in part by sodium lactate infusion.

Uric acid excretion was reduced in toxemic patients, as the result of enhanced tubular reabsorption from 91.5 to 96.3 per cent of the filtered load (77). Concomitantly, plasma uric acid increased from a normal of *ca.* 3.8 mg. per cent to 6.9 mg. per cent in toxemia. Benemid caused the excretion to increase to a greater degree in these subjects than in normals, manifesting an enhanced sensitivity to Benemid, according to the authors.

THE ROLE OF ANTIDIURETIC HORMONE IN THE REGULATION OF WATER AND SOLUTE EXCRETION

Mechanism for stimulation of release of ADH.—This topic has been recently reviewed by Thorn (3) and Verney (78), and emphasis is still placed by them on the changes in osmolarity of the blood supplying osmoreceptors in the hypothalamus. This mechanism has been reconfirmed by injection of 3 per cent NaCl into the carotid arteries of rats (79). Verney has localized the osmoreceptors by techniques of hemispherectomy and differential ligation of arteries supplying the hypothalamic areas to a zone in the anterior hypothalamus or preoptic areas contained in a volume of about 100 c.mm., supplied by the internal carotid artery, anterior and middle cerebral, and posterior communicating arteries that arise in the neighborhood of the carotid trifurcation. Some support for the idea that the supraoptic and paraventricular nuclei presumably manufacture ADH and transport the substance along the axons to the neural division of the pituitary, where it is stored, was supplied by Campagna *et al.* (80). They created incomplete lesions of the neurohypophysis of dogs by partial section of the pituitary stalk, partially disrupting the hypothalamico-hypophysial tract, but not damaging the hypothalamus or medial eminences. Water retention during a standardized water-load test was manifested on the sixth to tenth postoperative day, a phase which they looked upon as one of increased release of stored ADH

during a period of degeneration of the portion of the neurohypophysis to which the axons had been severed.

A number of reflexes which either inhibit or promote ADH release are cited, as well as drugs which have an antidiuretic effect [(3), see also (81 to 84)]. Papper *et al.* dispute the traditional action of morphine, presumed to stimulate ADH release, on the basis that when given to man, there was little change in urinary concentration of Na, K, and Cl as urine volume declined. Usually, a decrease in GFR as the result of a direct effect on the renal circulation was noted, and to this they attribute the antidiuretic effect. But if this is so, it is surprising that urinary concentration did not increase with the decrease in GFR.

Effect of ADH on tubular reabsorption of water.—The decrease in urine volume occurring after injection of ADH to a hydrated mammal has long been known. Brunner *et al.* (85) have reviewed the mechanism in rats, in which a maximal diuresis of 8.72 ml./rat/hr. was reached in *ca.* 54 min. after a standard load of 18 ml. of water. Concurrent studies on degree of hydremia were made (86). It is of considerable interest that removal of one kidney and subsequent removal of one-third of the remaining kidney had no influence on the water diuresis. Only when five-sixths of the total renal parenchyma was removed was there a depression of water diuresis. This depression disappeared after 20 days as the result of compensatory hypertrophy of the remaining kidney. They concluded that the rate of water diuresis did not appear to be restricted, within limits, by the amount of renal parenchyma. A complete answer to this observation would require studies of the filtration forces in the residual renal tissue.

In the past year the mechanisms involved in the diluting-concentrating process of the kidney have continued to receive wide attention. It might be well at this point to review again briefly the two approaches used in clarifying these processes. One, based upon the use of clearance techniques, has been reviewed by Smith (87). In brief, the main processes believed to occur in the kidney affecting water and sodium (the predominant solutes) are: (a) active reabsorption of sodium in the proximal tubule with passive reabsorption of water in maintenance of the isosmotic state; (b) further active reabsorption of a fixed amount of sodium in the distal tubule (T_{Na}^d) and of water, maintaining isomosticity in the presence of a maximum dose of ADH but not when smaller amounts are circulating (in the latter case, dilution of the urine ensues); (c) an active reabsorption of a fixed quantity of water ($T_{H_2O}^e$) in a more distal segment, possibly the collecting ducts. The magnitude of the $T_{H_2O}^e$ may be determined by examining an individual in the hydropenic state with or without the supplemental infusion of vasopressin, or in the hydrated state supplemented by vasopressin. When the urine flow is varied over a substantial range by infusion of an osmotic diuretic such as mannitol, it is found that above a critical volume (5 to 7 ml./min.), $T_{H_2O}^e$ reaches an approximately constant value ($T_m^e H_2O$) averaging about 5 ml./min. per 100 cc. of glomerular filtrate in man and dog.

The micropuncture studies of Wirz (88, 89) have promoted another approach to the understanding of the dilution-concentrating processes. He showed that: (a) during water diuresis the urine in the proximal tubules of the rat kidney is isotonic and that in the distal tubules is hypotonic, and (b) during endogenous antidiuresis the urine in the proximal tubule is still isotonic, but in the distal tubules it is hypotonic at their beginning and isotonic at the end. Previously Wirz and his associates (90, 91) found a steady rise in the osmotic pressure of the contents of Henle's loops, the capillaries, the extracellular fluid, and the collecting ducts from the corticomedullary boundary towards the tip of the papilla. This was explained on the theory of the hairpin countercurrent system. The establishment of these gradients was suggested as being brought about by some active cellular transport mechanism either drawing water from the descending to the ascending limb or transporting solutes in the opposite direction, or by a combination of these. As the collecting ducts pass through these hypertonic surroundings, water could be abstracted permanently from their lumen. In this view, ADH would need to be present for the establishment of the hairpin countercurrent system, changing the permeability to water of the descending limb of Henle's loop, the distal convoluted tubules, and the collecting tubules.

Hollander *et al.* (92) observed the effect of varying the rate of pitressin infusion from 0 to 15.5 mU/hr. on the free water clearance in man. This showed a marked decrease in proportion to dosage, in four instances reaching negative values. The data relating ΔC_{H_2O} to pitressin dosage were examined in a series of regression equations. The one which offered the most physiologically significant expression of the data was of the form $1/\Delta C_{H_2O} = A + B(1/\text{vasopressin rate})$. It was their belief that such an equation predicted, at increased rates of infusion of vasopressin, that ΔC_{H_2O} would approach a maximum asymptotically, inferring that the action of ADH is limited by a maximum rate. They speculated that each molecule of ADH is potentially able to combine reversibly in some way with a "site" in the renal tubular cell or on its membrane, thereby creating a "pore" through which water could flow at a constant rate. It is understood that the term "pore" merely indicates the rate-limiting element of the process. It is further assumed that the total number of potential "pores" is constant, that all sites have the same affinity for ADH, and that the rate of movement of water is proportional to the number of "pores". Sawyer (93), examining the influence of pituitrin on free water clearance in the toad, arrived at similar conclusions with regard to the regulation of tubular epithelial permeability to water, viewing the process as one in which the hormone acts to dilate "submicroscopic pores", thereby facilitating osmotic inflow of water, in analogy to its action on anuran skin and bladder. Orloff *et al.* (94) similarly conclude that vasopressin affects tubule membrane permeability in a graded fashion throughout the distal segment, both in the area where free water is present and in the terminal concentrating site.

Berliner & Davidson (95) present evidence that the final change to hyper-

tonicity of the urine may not be entirely dependent on ADH. In dogs engaged in maximal water diuresis, the arterial pressure was lowered to one kidney by means of a cuff around the renal artery. With the decline in filtration rate, urine osmolality greater than 300 mOsm. per kg. H_2O was regularly observed. The most important effect of the decrease in filtration rate was a limitation of the amount of Na and Cl which contributed to the formation of solute-free water in the distal nephron. In addition, because of the small amounts of solute delivered to the distal system with reduced filtration rate, an increased fraction of solute-free water escaped from the tubular lumen (a diffusion gradient for water reabsorption operates). Thus, a greatly reduced volume of fluid, including minimal amounts of solute-free water, was delivered to the final segment of the nephron presumably responsible for elaboration of the hypertonic urine, making it necessary for the removal of only a small volume of fluid to achieve concentration. This apparently went on in the absence of ADH. Observations by Kleeman *et al.* (96) in patients with diabetes insipidus were in entire conformity with the results of Berliner and Davidson. Reduction in GFR resulting from reduction in arterial blood pressure resulting from action of hexamethonium and tilting to 45° brought about an increase in urine osmolality from 50–75 mOsm./l. to 573–763 mOsm./l.

Although no mechanism for the final concentration of the urine under these circumstances was offered, if Wirz's hairpin theory is assumed to be correct, the stratification might be established by secretion of Na ions into the descending part of the loop of Henle, a suggestion made by Thorn (3). If the epithelial cells of the collecting ducts are freely permeable to water, there would thus exist a mechanism able to concentrate the urine in complete absence of ADH.

For an excellent summary of the latest views on the dilution and concentrating processes, the reader is referred to the aforementioned article by Berliner *et al.* (15). This somewhat modified version of Wirz's hypothesis emphasizes an active sodium transport out of the water-impermeable loop of Henle, which dilutes the tubular contents and creates the hypertonicity of the medullary interstitial fluid. This version also introduces the concept of a countercurrent flow of blood in the medulla to provide a way in which the concentration of diffusible substances placed in this region may be increased considerably above what might be expected from the flow of blood per se. It thus makes it possible to discount the blood flow as competitive with the urine for the increased osmotic pressure produced in the medulla by the sodium salts deposited by the loops of Henle.

Factors which modify ADH activity and the concentration-dilution process.—Dehydration for three days in human subjects produced an average $T_m^c H_2O$ of 6.7 ml./min., 65 per cent higher than that observed in the same subjects when fluid was forced (97). Inulin clearance averaged 104 ml./min. in the dehydrated vs. 110 in the hydrated state. Since similar results could not be achieved with injections of pitressin, the adjustment concerned something other than that resulting from prolonged exposure to ADH, and was at-

tributed to the state of hydration of the body tissues, including specifically the distal and collecting tubules. The observations of Grande *et al.* (98) on men on restricted water intake with low caloric intake, and accompanied by work, showed that the major adaptations for water conservation included not only reduction in both insensible water loss and rate of sweating, but marked renal conservation of water accompanying excretion of a very concentrated urine. It was calculated that significant water retention occurred in excess of the calculated retention of electrolytes, suggesting increased $T_m^c H_2O$. The observations of Dicker (99) on the urinary concentration in the rat during acute and prolonged dehydration are introduced here to remind of the complications of interpretation which may be added by reductions in filtration rate during dehydration. The effects of prolonged overhydration in humans were studied by De Wardener & Herxheimer (100); under this circumstance the kidneys manifested a reduced ability to concentrate the urine under conditions of infusion of pitressin, mannitol osmotic diuresis, and dehydration. This was correlated with an expansion of extracellular fluid volume combined with a paradoxical increase in plasma Na concentration, although total plasma osmolality fell. The mechanism is again elusive. The rise in plasma Na was achieved without a positive Na balance, suggesting transfer of Na into the extracellular fluid. They suggest that changes in the electrolyte composition of the tubule cells were finally responsible.

The effect of protein feeding and urea intake in man was examined by Epstein *et al.* (101). The $T_m^c H_2O$ increased from 5.3 to 7.2 ml./min. on a high protein and high urea diet. Concurrently, the ratio of urine to plasma osmolality rose from 2.74 to 3.32. The C_{IN} increased slightly from 107 to 113 ml./min. Low protein diet supplemented with 20 gms. of NaCl did not cause these changes; thus increases in solutes caused by electrolytes did not have the same effect on concentrating power as the solute derived from protein. Chronic administration of protein and urea promoted an adaptive response by the renal tubules by which water was conserved more efficiently and renal concentrating ability augmented. Other papers also discussed dietary aspects on urine concentrating ability (102, 103). Potassium depletion in rats reduced the tubular responsiveness to ADH (104). Lesions of the collecting duct which may relate to a decrease in $T_m^c H_2O$ (105, 106) have been described. Cortisone and hydrocortisone in man increased the rate of maximal urine flow during water diuresis, with little change in osmolal clearances, so that the increase chiefly resulted from free water clearance (107). An action on the renal tubule is suggested, since the response could not result from further ADH inhibition. The mechanism could thus be either caused by increased delivery of isotonic fluid of the distal system, and increased distal reabsorption of solute without water or, alternatively, a decreased osmotic permeability to water so that less water leaves the tubular lumen in response to the osmotic gradient, an action analogous to decreased ADH activity. Other pertinent papers compare the effects of vasopressin and oxytocin on water and salt excretion in hypophysectomized rats (108), the

mechanism of ethanol action (109), neurogenic stimulation of ADH release (110), and the effect of adrenalectomy on the water concentrating process (111).

Additional reports which can be appended to this section include an investigation of the plasma level of ADH in humans in relation to dehydration and various types of water loading (112) and a study of the plasma ADH activity of marsupials during exposure to heat (113). Three papers (114, 115, 116) are concerned with the metabolism of ADH in the liver and kidney. The kidney demonstrates a surprisingly high rate of inactivation of ADH. A method for assaying small amounts of ADH is described (117).

Intratubular hydrostatic pressures.—It appears pertinent to interpolate at this point the findings of Gottschalk & Mylle (118) who investigated by micropuncture the pressures in rat renal tubules and peritubular capillaries. The hydrostatic pressure in the proximal tubules averaged 12.5 ± 2.2 mm. Hg, and in the distal tubules 6.7 ± 1.6 mm. During osmotic diuresis, the pressure in both the proximal and distal tubule increased, the pressure difference disappeared, and, in general, a linear relationship was found between distal intratubular pressure and the rate of urine flow. This was taken to indicate a relatively constant resistance to flow beyond the distal tubules, i.e., in the collecting system. The disappearance of the pressure difference between proximal and distal tubules indicates that the loop of Henle must also dilate. Pressure in the peritubular capillaries agreed with the proximal tubular pressure. This was thought to result from compression of the capillaries by the dilated tubules and by compression of the thin walled interlobular veins and arcuate veins.

The collecting tubules may also be distensible in part; the fact that pressure-flow curves extrapolate to positive values suggests that the collecting tubules are either compressed by an external force or have a "critical opening" pressure. Direct observation of the tubules in rats during osmotic diuresis by Brunner *et al.* (119) has confirmed the above observations and added the fact that small collecting tubules dilate during diuresis. The greatest change in diameter occurred in the proximal tubule.

THE REGULATION OF ALDOSTERONE SECRETION

The manifest importance of this hormone in electrolyte handling by the kidney merits considerable attention to the mechanism of regulation of its output by the adrenal gland. It is apparently secreted primarily by the zona glomerulosa of the adrenal, whereas the other steroids are formed by the inner zones. Its synthesis by the glomerulosa tissue has been demonstrated by *in vitro* studies (120 to 124). The secretion of aldosterone is relatively independent of the pituitary. Thus, the hypophysectomized rat is able to vary the output of the steroid in response to change in electrolyte intake (125, 126). The hypophysectomized dog secretes aldosterone at near-normal levels, whereas the secretion of the other steroids declines to the expected low rates (127, 128, 129). Patients with panhypopituitarism have normal levels of uri-

nary aldosterone (130), and following hypophysectomy in man urinary aldosterone levels appear to remain normal (131).

Nevertheless, some type of humoral (tropic) control of the secretion of the glomerulosa is indicated (132), and there is some evidence that it arises from the head (brain substance) of the experimental animal (133). Thus, in the decapitate animal, the output of the steroid falls profoundly, but if the major vascular connections of the head are left intact, and the dissection is otherwise the same as in decapitation, output of the steroid is quite normal. Extracts of beef diencephalon stimulate aldosterone secretion (134), but such extracts are not devoid of corticotropin activity, suggesting the presence of ACTH in these extracts. A protein fraction isolated from urine appears to be a specific factor for the stimulation of aldosterone synthesis by the rat adrenal *in vitro* (135), and may be the "glomerulotropic" hormone. Orti *et al.* (136) also found, in the urine of adrenalectomized rats deprived of salt, a substance which was thermolabile at pH 7, extractable with butanol, and which did not dialyze through a cellophane membrane. This stimulated the release of aldosterone in hydrated, intact assay rats. The latter authors supplied evidence that the substance is not ACTH.

Despite the aforementioned evidence suggesting a lack of pituitary-dependence for aldosterone secretion, conflicting evidence appears in reviewing the effects of ACTH. It increases the output of aldosterone in rats (125, 126), but much less so in the dog (128), based on adrenal vein blood analysis. Urinary aldosterone studies following response to ACTH are not consistent: some have found transient increases (137 to 140), while others have found no effect (141 to 144). Of the separate purified fractions of ACTH, δ_1 -corticotropin proved several times more potent in stimulating the release of aldosterone than β -corticotropin (145). In view of what has been said about the lack of regulatory role of the pituitary, this becomes an enigma, unless the fraction is diencephalic factor stored in the pituitary.

That the nervous system plays an important role in electrolyte metabolism has been emphasized in several reviews (9, 146, 147). It has raised the challenging question of the existence of regulating centers which may ultimately control aldosterone output. The complexity of interpreting clinical data has again been re-emphasized (148). Experiments involving hypophysectomy, decapitation, severing of nerve connections between the head and trunk, decortication, or decerebration were carried out in dogs (128, 129, 133), combined with isolation of steroids in adrenal venous blood. These experiments showed that removal of the cerebral cortices did not decrease aldosterone output, but that subsequent removal of the brain substance rostral to the corpora quadrigemina resulted in a profound decrease in the steroid output. The conclusion was reached that aldosterone was regulated by a structure probably located in the diencephalon. More precise localization has been made available by the technique of placement of coagulation lesions (149, 150). The latter workers, using cats as the experimental animal, were able to show that, when the central core of the caudal diencephalon and

rostral midbrain were destroyed, secretion of both aldosterone and hydrocortisone was suppressed (adrenal vein blood analysis). When the lesions extended into the rostral pons, a significant increase in aldosterone output was noted, suggesting the possibility of an inhibitory center. Daily & Ganong (149) found that ventral hypothalamic lesions in dogs produced diabetes insipidus, gonadal atrophy or inhibition of hydroxycorticoid secretion or both, but none of the animals showed abnormal sodium and potassium metabolism, and all withstood salt restriction and handled a salt load in a normal fashion. It is entirely probable that their lesions did not cover the more dorsal areas destroyed by Newman *et al.* (150).

Again emphasizing the role of aldosterone as the important sodium and potassium regulating hormone operating on the kidney, although admittedly not the only one (146), it is important to consider the factors which influence aldosterone output, presumably through mediation of the brain centers. Thus, reduction of sodium intake appears to be an important stimulus to aldosterone secretion (139, 147, 151 to 154) although it is not clear whether this is the result of decrease in the sodium concentration of the extracellular fluid or contraction of the extracellular fluid volume, possibly triggering volume receptors of some sort (155, 156, 157), although Johnson *et al.* (153) insist that very small changes in plasma and extracellular fluid volume occurred in their subjects with combined Na and K deprivation. Insignificant changes in output of 17-ketosteroids and 17-hydroxycorticosteroids occurred, indicating that corticotropin played no part in the increased aldosterone output.

Although Na deprivation played the dominant role in the above study (153), there was a suggestion that decreased K intake favored a decreased aldosterone output into the urine. There is other evidence that K may have a regulatory role (132), depletion causing a decrease in aldosterone output (125, 126) in the adrenal venous blood, while K loading is associated with an increase in aldosterone level of the urine (158). Muller *et al.* suggest that the effects of K are mediated by loss of body water (hence via volume receptors) following K administration (159), but Laragh (158) feels that the ion may have a direct effect on the regulatory mechanism; however, this mode of operation is not clarified.

Changes in body fluid volume as a regulatory mechanism for electrolyte and water exchange by the kidney, presumably via changes in ADH and aldosterone output, have been the subject of much research in the past [for reviews, see (9, 146, 147 and 160)] and continue to attract attention. Final mechanisms are not understood, but the evidence is undeniable that variations in aldosterone do occur with changes in body fluid volume, based upon urinary levels of electrolyte or urinary steroid (139, 154, 155, 156). Fine *et al.* (161) have shown that phlebotomy of 450 to 670 ml. in man is followed by a decrease in Na excretion and urine volume, no change, or a slight rise in K excretion, and an increase in aldosterone in urine (bioassay). When the lost blood was replaced by 25 per cent albumin, changes were less consistent;

and when hemodilution was corrected by saline infusion to circumvent withdrawal of fluid from the interstitial compartment, no significant change in any function was observed. They conceive that the rapid uptake of isotonic fluid from an extravascular locus, probably in the interstitial space, proves a stimulus which is monitored by some suitable local receptor. Its location is not defined.

A different type of mechanism is suggested by the work of Davis, Pechet & Ball (162). In dogs with thoracic inferior vena cava constriction or experimental right-sided congestive heart failure (produced by pulmonic stenosis), aldosterone secretion into adrenal vein blood increased from a normal of 2.7 $\mu\text{g.}$ per hr. to 11.6 and 16.5 $\mu\text{g.}$ per hr. respectively, accompanied by salt and water retention and development of ascites. They describe the order of events as follows: elevated venous pressure, extravasation of fluid and electrolytes, adrenal cortical stimulation, increased secretion of Na-retaining hormone, and Na retention. The constriction needs to be applied above the liver to favor maximal extravasation of fluid from the hepatic vascular and lymphatic channels. Constriction above the renal veins and adrenolumbar veins does not produce an effective chronic stimulus to sodium retention despite maintained pressures of 120 to 122 mm. H_2O (163). Thoracic inferior vena cava constriction in hypophysectomized dogs produced sodium retention and ascites as in normals, accompanied by increased excretion of aldosterone into the urine (164). The expected atrophy in the two inner layers of the adrenal, the fasciculata and reticularis, occurred; but the glomerulosa, if anything, hypertrophied, supporting the idea that the release of aldosterone is not solely dependent on the pituitary hormones. In an interesting manner it was demonstrated that when the usual ascites formation was minimized by the placement of a cast about the abdomen, to reduce filtration of fluid into the peritoneal cavity during inferior vena cava constriction, aldosterone output was decreased with a concurrent increase in Na excretion (165). A clinical corollary of the animal experiments is the finding that the usually high urinary aldosterone in portal cirrhosis with ascites does not occur in cirrhosis without ascites nor in biliary cirrhosis (166).

The work of Davis *et al.* does not explain the casual relationship between the episode of fluid extravasation by the liver capillaries and lymphatics and the ultimate modification of the central regulating mechanisms. Further complication of interpretation is added by the inability of Driscoll *et al.* (167) to confirm the work of the above cited group. Congestive heart failure produced in dogs by pulmonary artery constriction resulted in no increase in aldosterone output, despite signs of ascites and pleural effusion accompanying the right-sided cardiomegaly. The obvious implication is that fluid accumulation proceeded in the absence of detectable alteration of aldosterone output. No immediate explanation of the differences is forthcoming.

Recent preliminary studies indicate that the degree of distention of the right atrium may play a role in aldosterone secretion (168). In dogs, when the right atrium was stretched (by external threads under tension in such a

way as not to disturb blood flow), the aldosterone secretion rate was less than one-half that of the controls or the rate when the left atrium was similarly stretched. The suggestion is that a reflex system exists, with receptors in the right atrium, perhaps parallel to the system proposed for ADH release (169). In operation, it might be assumed that with an increase in venous return associated with increase in body fluid volume a decrease in aldosterone output would result or, as a corollary, an increase in aldosterone secretion might be the result of a fall in atrial pressure such as occurs with hemorrhage, resulting in salt (and water) conservation. Henry & Pearce (169) have described an atrial type B fibre in the dog which responds to inflation with a balloon with burst of activity on the oscillograph recording potentials from the vagus nerve. Associated with the rise in pressure is a diuresis; the response is blocked by cold narcosis. The workers are emphatic that the volume, rather than pressure, is the important stimulus since bursts of activity are minimal during the *a* wave of the atrial cycle, at a time when the pressure is highest.

As a concluding remark for this section, it seems fitting to suggest that the experiments described above concerned with thoracic inferior vena cava constriction might have their explanation in an atrial volume receptor mechanism. Thus, restriction of venous return and the resultant fall in atrial pressure would result in increased aldosterone output via the diencephalic regulatory centers. The immediate integration of the findings of McCally *et al.* (168) and those of Henry & Pearce is not evident; the latter subservise ADH regulation, with increased activity apparently inhibiting ADH release via hypothalamic centers. Similar receptors and afferent activity may likewise be inhibitory to the centers controlling aldosterone release. As Bartter has recently concluded (170), the most fruitful approach to the understanding of the mechanism controlling aldosterone secretion lies in the regulatory aspects of the intravascular volume, and with it the likely possibility that receptors for such regulation lie herein.

ELECTROLYTE EXCRETION

Influence of hemodynamic factors.—The significance of changes in glomerular filtration rate, which occur directly or indirectly as the result of renal hemodynamic alterations, on excretion of NaCl has been thoroughly reviewed by Wesson (10), and elaboration in the current review hardly seems necessary. The topics he considers under filtration rate changes are: effects of changes in renal arterial and venous pressure, and in ureteral pressure; action of pressor and depressor drugs; denervation; diurnal variation; salt and water loading effects; salt deprivation; acute blood sequestration; effects of exercise; and finally, the contribution of GFR effects to electrolyte excretion in the clinical conditions of heart failure, nephrosis, and cirrhosis in man. Although not neglecting the consideration of tubular factors arising from endocrine and other influences, he states (p. 365):

The pervasiveness of correlation between filtration and salt excretion, irrespective of the degree to which it may be spurious, has not been cited to prove that changes in

salt excretion are caused by changes in filtration, but rather to demonstrate that experimental support for the alternative thesis, that excretion changes result from alteration of tubular function, cannot yet be found. Therefore, until experimental procedures are substantially refined, we have no basis for viewing acute changes in salt excretion as more than complex loading effects.

Although the above opinion is sweeping in its scope, a re-emphasis of the role of GFR in electrolyte excretion certainly seems justified. Current papers dealing with diurnal variations in Cl excretion (171), and influence of posture and hydration (172, 173) have appeared without adequate measurement of GFR.

Recent studies which extend topics discussed in the above review include the physiological response to saline infusion (174), the effects of exercise in hypertensive subjects (175), effects of changes in posture (176), the influence of autonomic vasoregulatory reflexes (177), the role of renal nerves and neurogenic influences on electrolyte excretion (178, 179, 180), the effects of positive pressure respiration (181), and the influence of mechanical reduction in arterial pressure (182). Of interest in the paper by Lauson & Thompson (182) was the finding that although total cation excretion diminished with decrease in GFR, the fraction neutralized by K, NH_4 , and H ions increased correspondingly. PAH and thiosulfate were given as unreabsorbable anions. Urine pH fell as GFR was reduced, and net "secretion" of K was demonstrated with increasing frequency as GFR was reduced below 60 per cent of control. Below 40 per cent, 29 of 41 ratios exceeded 1.0, with the highest value at 2.96.

The difficulty in separating hemodynamic factors from specific tubular effects is illustrated by the effect of pentobarbital anesthesia on electrolyte excretion (183). Its prolonged effect is to produce renal vasoconstriction favoring electrolyte retention. When pentobarbital is, however, injected directly into one renal artery (the opposite serving as control), a significant increase in Na excretion occurs over the control, despite a small decrease in GFR suggesting a direct influence on the tubular epithelium. No effect was noted on K excretion, however. Similarly, although both l-epinephrine and l-nor-epinephrine (184, 185) induce intrarenal vasoconstriction, and hence may influence electrolyte excretion on this basis, the possibility of direct tubular activity cannot be overlooked. Thus, tubular reabsorption of Na, K, Cl, and bicarbonate-obligated cation increased without consistent change in inorganic phosphate, ammonium excretion, urinary pH, or titratable acid (185). Fluctuations in GFR obtained, but the authors contended that the pattern of electrolyte alteration was more than the corresponding changes in GFR. As an alternative to local intrarenal circulatory adjustments and reduction of RBF to tubular tissue, they considered the possibility of a direct action through alteration of tubular metabolism.

Tubular factors: site of hormone action.—An earlier section has devoted considerable space to hormonal factors concerned with electrolyte handling. An attempt to localize specifically the site of action of sodium-retaining

hormones in the nephron has been made by Nicholson (186). What was presumed to be specific proximal tubular damage, confirmed by histological studies, was created by the infusion into the renal artery of racemic sodium tartrate. Distal tubular impairment was created by retrograde perfusion of HgCl_2 , again confirmed by typical histological alterations. The findings were that when the damage was in the proximal tubule, neither deoxycorticosterone nor aldosterone appeared to decrease Na excretion in doses that caused reduction in normal animals. The distal tubular impairment had no effect on the ability to reabsorb Na, which proceeded normally in the presence of normal proximal tubular tissue. Thus, the chief site of action, according to Nicholson, of these hormones is the proximal convoluted tubule. There was no observed effect on K handling.

Tubular factors: influence of solute loading.—In a complex analysis of the effects of varying urea loads and mannitol on solute excretion in hydrated, hydrated and acidotic, and hydropenic dogs, West & Bayless (187) observed a three- to fourfold increase in solute excretion including increased excretion of Na and Cl, without an effect on the partitioning of urine with respect to Na and Cl. Effects were more variable in the acidotic and dehydrated animals than in the hydrated controls. Potassium excretion was not influenced in the hydrated dogs, but increased in the hydropenic and acidotic (hydrated) dogs. Generally, the ratio of NH_4 , P, HCO_3 , and acid to total solute excretion fell with solute loading. The authors believe that the tubule regulates the concentration of electrolyte (Na, Cl) at some critical point of the nephron, as a function of the volume of the fluid at that point (volume varied by the degree of osmotic diuresis) and in turn the presenting concentration of electrolyte. Their analysis places the site in or perhaps proximal to the ascending limb of the loop of Henle. Mannitol osmotic diuresis in dehydrated, acidotic human subjects (188) produced a fall in urinary pH, and base sparing, resulting from some specific but apparently not well understood influence on the distal tubular H^+ secretion mechanism.

Acid-base regulatory influences.—Nicholson (189), by the aforementioned technique of proximal tubular damage by unilateral infusion of racemic sodium tartrate, showed that in acidotic dogs there was a definite decrease in titratable acid and H^+ concentration in the urine of the nephrotic kidney, as determined by dye indicator studies. According to his belief, the proximal tubular acid-base regulating mechanism appears to be engaged maximally when the animal is excreting a markedly acid urine. On the other hand, when the acidity of the urine is slight, acidification of the urine appears to take place in the distal tubule. The absence of an acid reaction in the proximal tubular cells when the urine is not highly acid suggests a "facultative" H^+ secretion.

Brodsky *et al.* (190), in the course of study of the effect of loading dogs with weak organic acids on the urinary pCO_2 , evaluated two schemes of urinary acid production, the bicarbonate ion reabsorptive mechanism, and the H^+ secretion mechanism. They found that when hydropenic animals were

loaded with lactic acid or β -hydroxybutyric acid, the CO_2 tension of the urine was greater than plasma in 106 observations, was less than plasma in 33 observations, and was equal to the plasma on 25 occasions. In the light of these observations, they consider both mechanisms. Limitations of the bicarbonate reabsorption mechanism are that it cannot account for the maximal rates of excretion of titratable acid, and it fails to account for the high urine pCO_2 observed in some samples of acidic urine. On the other hand, the H^+ secretion mechanism, although it can account for the higher CO_2 urine than plasma CO_2 , cannot, according to the authors, account for the production of urine where the pCO_2 is less than that of the plasma even when fortified by assumptions of a "trapping" process. They conclude that it is necessary to invoke the presence of at least two separate mechanisms to account for all the pCO_2 gradients observed during the production of acidic urine.

Richterich *et al.* (191), in their recent studies of ammonia production in the guinea pig and rabbit, make the novel observation that ammonia can be excreted into an alkaline urine. Apparently this is an adaptation in species which normally excrete an alkaline urine. In acute studies involving saline, bicarbonate, or HCl administration, a depression of ammonia excretion was observed when the urine pH was close to neutrality, but increased again at acid pH as in dog, man, and the rat.

The influence of acute changes in acid-base balance on renal calcium excretion has been investigated by Williamson & Freeman (192). They found a linear relationship between the amount of calcium reabsorbed and the load in the range studied (*ca.* 120 to 470 m.eq./min. $\times 10^3$ of Ca filtered), and no evidence of a calcium T_m in this range. The state of acid-base balance apparently had no influence on this relationship. It should be added that at high loads, although reabsorption increased with load, the per cent reabsorbed, so that urinary excretion increased.

Miscellaneous observations on electrolyte excretion.—The effect of sodium depletion on plasma K concentration and the capacity of the kidney to excrete K has been examined (193). K-excretion in depleted dogs was quantitatively similar to that of the control animal. Tubular secretion was demonstrated in severe Na depletion, and reduction of available Na could not be made a limiting factor for K excretion. Protamine chloride causes a marked decrease in per cent of K reabsorbed by the rabbit kidney (194). Also, when K secretion was established by K loading, the secretion was depressed, e.g., from a ratio of 1.21 to 0.83. The mechanism is not understood, although protamine strongly inhibits active accumulation of K by kidney slices.

Cortisone has been shown to increase the urinary excretion of PO_4 in humans and dogs by depressing its tubular reabsorption (195). The latter conclusion was reached because its action was unaccompanied by elevation of serum inorganic PO_4 . Unfortunately, no measurements of GFR were made, so that the possibility of an increase in GFR causing the effect must be considered. Calcium-gluconate or lactate infusion into man and monkeys was shown to cause an increase in Na, Cl, and water excretion (196). The mecha-

nism of action is not known; changes in GFR and imposition of an osmotic load were ruled out. Potassium excretion was increased by such infusion less regularly in man than in the monkey, because the latter is said to be almost entirely on a salt-free diet. Thus, in this instance, if an anion load is imposed by Ca-induced chloruresis, K is excreted because less Na is available. Phosphate clearance tests have been employed as a means of diagnosis of parathyroid dysfunction (197, 198).

Some interesting observations on comparative physiology of electrolyte excretion are here appended. Berglund & Forster (199) investigated the renal tubular transport of inorganic divalent ions by the aglomerular marine teleost, *Lophius americanus*. Maximum rates of tubular excretion were obtained for Mg, SO_4 , and S_2O_3 . Elevation of MgCl_2 levels of the plasma markedly depressed Ca excretion; S_2O_3 similarly depressed SO_4 excretion. Experimental evidence suggested a transport system for divalent cations distinct from that for divalent anions. Neither system was influenced by Benemid. Carinamide depressed SO_4 and S_2O_3 excretion, with no effect on excretion of Mg and Ca.

Tubular secretion of PO_4 was demonstrated by infusion into the portal system of the hen (200). This has also been demonstrated in the sculpin, dogfish, and goosefish. Parathyroid extract was shown to promote tubular secretion of PO_4 in the hen.

When cormorants are fed fresh fish, the water content of the fish is more than adequate for renal elimination of salts and nitrogenous products. With salt loads, an extrarenal mechanism is brought in: a highly hypertonic fluid [500 to 600 m.eq./l. for both Na and Cl] drips from the external nares (201). The rate of secretion may be up to 0.2 ml./min. in a 1.5 kg. bird, and this contains practically only Na and Cl. The production of the nasal secretion can be stimulated by a nonelectrolytic osmotic load (e.g., sucrose).

ACTION OF DIURETICS

Kessler, Lozano & Pitts (202) [see also (16)] have studied the structural relationship to diuretic activity of certain organic compounds of mercury. Of 13 compounds studied, six had diuretic properties and seven did not. All were accumulated in the kidney of dogs, but no relationship between pattern of distribution or renal concentration and diuretic activity could be established. The effects bore no relationship to rate of excretion. Three of the compounds most widely used as inhibitors of sulfhydryl enzymes *in vitro* were devoid of diuretic activity. Therefore, diuresis did not seem to be related to inhibition of sulfhydryl enzymes. By exclusion, they decided that diuretic activity was related to structural configuration: (a) a chain of not less than 3 C atoms; (b) an atom of Hg attached to the terminal C of this chain, and (c) some hydrophylic group not less than 3 C distant from the Hg, suggesting to them a "critical lock and key" relationship to the site of mercurial action.

Exonerating changes in the succinoxidase system and sulfhydryl systems as being involved in the enzymatic basis for mercurial diuresis, Greif &

Jacobs (203) examined the effects of Hg^{203} labelled chlormerodrin on the P/O ratio (moles of inorganic phosphate esterified to oxygen atoms consumed) of rat kidney mitochondria. In intact rats, doses 20 times greater than required to produce a diuresis failed to alter function of subsequently isolated mitochondria, but in no instance was there sufficient mercury bound to these structures to exceed the amounts necessary to lower the P/O ratios observed *in vitro* studies. Thus, they conclude that there is no evidence that the mercurial diuretics impair the ability of the mitochondria to carry out oxidative phosphorylation in the range able to produce diuresis. Heinemann & Becker (204) observed an increase in free water clearance as the result of action of mercurhydrin. They concluded that the mechanism resulted from the inhibition of solute reabsorption (mostly Na and Cl) proximally; the increased solute loss carried water to the more distal site of free water elaboration.

Surtshin & Parelman (205) found that rats given an aminonucleoside related to puromycin developed proteinuria, hypoproteinemia, edema, and ascites, but became more resistant to mercuric chloride. Cause of the protection was not known, but the suggestion was made that the increased albumin in the urine blocked mercury reabsorption and increased Hg excretion by binding with protein lost in the urine. Rats on a low protein (sucrose) diet are also protected (206, 207). Beyond speculation that the increase in resistance to mercury toxicity may be related to a decrease in binding of Hg by sulfhydryl groups of renal nuclei and mitochondria, no good explanation of the protective action was offered.

The new diuretic agent chlorothiazide (6-chloro-7-sulfamyl-1,2,4-benzothiadiazine-1, 1-dioxide) acts more like an organomercurial than a carbonic anhydrase inhibitor type of diuretic agent, according to Beyer *et al.* (208), increasing preponderantly the excretion of Na and Cl [see also (209, 210)]. The excretion of HCO_3 and K is normally increased slightly, if at all. It is orally effective in treatment of edema of various etiologies. Laragh *et al.* (211) found it caused an increase in urine volume as the result of increase in solute and electrolyte excretion with no change or even slight reduction in free water clearance. Another agent also differing from acetazoleamide and acting like chlorothiazide in promoting Cl loss rather than HCO_3 loss is dichlorophenamide (1,3-disulfamyl-4,5-dichlorobenzene) (212).

Seldin *et al.* (213) followed the effects of prolonged (10 day) administration of acetazoleamide (Diamox; 2-acetyl-amino-1,3,4-thiadiazole-5-sulfonamide) on the excretion of acid and the carbonic anhydrase and glutaminase activities of the rat kidney. Presumably, complete and continued inhibition of carbonic anhydrase was produced, and all rats developed a severe acidosis, and also marked hypernatremia and hyperchloremia. An initial moderate fall in urinary NH_3 occurred but was restored when NaCl was given. When only KCl (4mM/8 hr.) was given, NH_3 production increased above the control, and this rise was associated with an increase in renal glutaminase. Their explanation was that the higher NH_3 excretion in rats given a diet free of

NaCl may be due to the intense stimulus to reabsorb Na, so that the unreabsorbed anion (acetazoleamide) was covered by increased secretion of ammonia.

The mechanism of an observed rise in CO_2 tension of the urine of dogs as the result of acetazoleamide action, Capeci *et al.* (214) state, is the result of the amount of nonbicarbonate buffer in the urine. When such buffer is present, the formation of H_2CO_3 from bicarbonate when acid is added is slowed and the resultant formation of CO_2 is delayed until the tubular urine reaches a segment relatively impermeable to CO_2 . Here CO_2 dissipation is prevented and the CO_2 tension of the urine rises as the system proceeds to equilibrium. They attribute this buffering action to acetazoleamide itself, a weak dibasic acid with strong buffer properties at pH 7 to 10 ($\text{pK}' = 7.47$).

Acetazoleamide infusion produced an increase in plasma Ca and PO_4 , along with increases in urinary excretion of these electrolytes (215). Since no effect was produced in nephrectomized or ureteral ligated dogs, the conclusion was obviously consonant with the view that the renal ion exchange mechanisms plays a role in the economy of calcium. Olewine & Perlmutter (216) compared acetazoleamide effects in intact as compared to adrenalectomized rats. The usual changes in excretion were seen, but the absolute increases in water and potassium excretion for the adrenalectomized rats were less than for the intact, while sodium excretion exceeded that of the intact group at the fourteenth day and subsequently. Coulson *et al.* (217) have made the interesting observation that acetazoleamide promotes an alkalemia rather than acidemia in the alligator, as the result of excessive chlorine urinary loss with little sodium loss. A continued potassium loss also occurs. The effects of chlorothiazide and dichlorophenamide were also investigated.

WATER AND ELECTROLYTE EXCRETION IN RENAL DISEASE

Several reviews have appeared on this subject; fluid and electrolyte disturbance in heart failure and their treatment (218, 219, 220), the nephrotic syndrome (221), the patterns of water and electrolyte change in injury, surgery, and disease (222), and on the K-losing syndrome (223).

A number of papers have appeared on electrolyte and water handling in hypertension. When hypertonic NaCl (5 per cent) is infused into hypertensives, the ratio of excreted to filtered sodium both before and during the infusion was significantly higher in the hypertensive than the normotensive groups (224). This was accompanied by a larger urine volume, as a result of the added osmotic effect of the sodium salt. The effect could be modified by Na restriction but was not reduced to normal. Cottier *et al.* (225) confirmed these findings, but noted also an increase in K excretion with the hypertonic NaCl infusion. Both groups found no correlation with changes in GFR caused by the saline infusion. The latter group found a good correlation between sodium clearance and total renal vascular resistance, and suggested that the basic arteriosclerotic processes occurring in the hypertensive kidney might be related in a causative fashion to water and electrolyte excretion.

(With severe arteriosclerotic changes, salt excretion fell off with the highest renal vascular resistances, apparently a result of significant reduction of GFR.) When blood pressure was reduced by antihypertensive drug therapy (hydralazine, reserpine, and hexamethonium) during saline infusion (226), there appeared to be a statistically significant reduction in the ratio of excreted to filtered sodium with comparable increases in GFR. The authors inferred some link between the blood pressure level and tubular function but offered no explicit mechanism. Urinary aldosterone excretion is known to go up in hypertension (227, 228), but this is probably a poor index of production (229). A strong claim is made for the contribution of the adrenal cortex to renal and renoprival hypertension (230). In experimentally hypertensive rats, a defect in the water conservation mechanism was uncovered (231). The $T_m^e\text{H}_2\text{O}$, which in normals averaged $10.9 \pm 3.0/100 \text{ ml./GFR}$, ranged in the hypertensives from 6.71 to 8.2, and was associated with a higher plasma osmolality. Albert *et al.* (232) compared serum Mg, Na, and K concentrations of essentials hypertensives with normals. Serum Na was claimed to be significantly higher in the hypertensives (147.7 ± 5.0 as compared to 142 ± 3.0); from this they argued in favor of an excessive adrenal cortical activity.

In sickle-cell anemia (233), $T_m^e\text{H}_2\text{O}$ tended to fall below the range of normal. A specific tubular defect is suggested by the low osmotic U/P ratio obtainable during oliguria; the value was 1.73 or less compared to the normal of 3.0 to 4.0. In Wilson's disease (hepatolenticular degeneration), multiple renal defects are seen as the result of deposition of copper in the kidney tubules which interferes with essential enzyme systems (234). Phosphate clearances exceed normal and are reflected by a low plasma inorganic PO_4 . There is evidence of impairment of the bicarbonate reabsorbing mechanism, since urine tends to be more alkaline than normal. However, the response to NH_4Cl acidosis is normal. Patients with hypercalcemia and attendant tubular damage show marked defects in $T_m^e\text{H}_2\text{O}$ (235). Urinary obstruction, when relieved (236), is followed by a period of salt and water wasting, the result of damage to the tubules. The differential diagnosis for psychogenic diabetes insipidus, marked by an impulse to drink water, is the much higher GFR than in true diabetes insipidus (237). The obvious need for rigid restriction of Na and water during the oliguric phase of acute renal failure is emphasized by the observed increase in total body water and extracellular water space (238).

WATER AND ELECTROLYTE TRANSFER IN OTHER TISSUES

An electrical potential makes the blood about 30 mv. positive with respect to the reticulorumen contents (239). Thus, Cl absorption from the reticulorumen sac takes place down its electrochemical gradient. It is concluded that the movement of Cl against its concentration gradient from the rumen contents into plasma occurs because of the potential difference between the two phases. Other related studies are concerned with Cl transport in the gastric mucosa of the frog (240) and ion and water fluxes in the ileum

of rats (241). Solomon applied similar techniques in measuring electrical potential across the rat's tubule (242). Random insertion of microelectrodes into tubules gave a bimodal type of distribution, with the lower means ranging from -19 to -39 mv., and the higher from -34 to -70 . It was suggested that the two groups of potentials arose from the proximal (lower) and distal convoluted tubules (higher potential difference). This was arbitrarily based on the more frequent occurrence of the former and assumption of greater accessibility of the proximal tubule. This was verified by the differential concentration of previously injected phenol red found in each segment. Temporary occlusion of the circulation caused the potential to swing toward zero, then return on restoration of blood flow. No attempt was made to elucidate the cause of the mechanism beyond the statement that it could be caused by several passive diffusion potentials or could be a reflection of the transport activity of the secretory cell sheet. It seems evident that a gap still remains between measurement of tubular potentials in the kidney and a definitive understanding of the transport processes of ions and other substances. On the other hand, such studies represent a logical progression from the studies of the forces involved in ion transport in other tissues, e.g., the frog skin (243 to 247), and may ultimately prove to be of extreme value in the resolution of mechanisms.

By passing catheters to the upper and lower end of the ureter in man (248), Garby *et al.* found evidences of electrolyte diffusion across the ureteral mucosa. The following decreases in concentration were noted: urea, 18 per cent; Na, 19 per cent; Cl, 15 per cent; and K, 25 per cent. The pH and CO_2 content were variable and appeared to indicate no definite trend. A relationship of the changes to rate of urine flow was observed, the more marked changes occurring at lower flow rates, confirming the diffusion mechanism. Although sodium and other electrolytes appear to be absorbed somewhat from the dog's bladder (249), this apparently does not occur in the human bladder (250). However, an active sodium transport was demonstrated by the isolated toad bladder (251). When I^{131} as NaI and Na^{24}Cl were installed into fowl's cloaca, and the blood checked for radioactivity, no cloacal absorption could be demonstrated (252). Despite the histological similarity to the large intestine, the cloaca has more the functional characteristics of bladder mucosa in the sense that absorption may be absent or small. Difficulties arising from ureterosigmoidostomy including hyperchloremic acidosis because of the more rapid absorption of chlorine than sodium from the large bowel, in exchange for HCO_3 , have led to a technique of urinary diversion to ileal loops or segments (253).

MISCELLANEOUS

Gagnon & Clarke (254) have studied renal function in the chimpanzee. Inulin clearance varied from 62 to 103 ml./min. M^2 . The creatinine to inulin ratio ranged from 1.24 to 1.51 and was depressed by further creatinine infusion, by PAH, and by Benemid. Renal function in the sheep foetus has

been ingeniously studied by Alexander *et al.* (255). Of interest was the finding of electrolyte (Na, K, Cl) reabsorption in excess of water between 61 to 130 days of gestation, thus producing a hypotonic urine. Excretion of phenol red and inulin by the foetal and newborn rabbit was studied by Levine & Levine (256). Surprisingly, the phenol red clearance was less than the inulin clearance before birth, and subsequently rose to a ratio of 2.0 by one month after birth. The effect was attributed in part to the higher degree of plasma binding of the dye in the foetus. Renal function during human pregnancy has been reinvestigated (257). The use of subcutaneous depots of inulin and PAH gives satisfactory clearance comparisons to the infusion technique in dogs, but the values in the human are inexplicably low (258). In the rat, T_mH_2O averaged 5.0 ml./100 ml. of GFR, a figure comparable to man and dog (259). Finally, an interesting investigation of the drinking mechanism in the goat by using the technique of electrical hypothalamic stimulation is described by Andersson & Wyrwicka (260).

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LITERATURE CITED

1. Darrow, D. C., and Hellerstein, S., *Physiol. Revs.*, **38**, 114 (1958)
2. Schmidt-Nielsen, B., *Physiol. Revs.*, **38**, 139 (1958)
3. Thorn, N. A., *Physiol. Revs.*, **38**, 169 (1958)
4. Heller, H., Ed., *The Neurohypophysis* (Butterworth Scientific Publications, London, Engl., 275 pp., 1957)
5. Andersson, B., in *The Neurohypophysis*, 131 (Butterworth Scientific Publications, London, Engl., 275 pp., 1957)
6. Brooks, F. B., and Pickford, M., in *The Neurohypophysis*, 141 (Butterworth Scientific Publications, London, Engl., 275 pp., 1957)
7. Wirz, H., in *The Neurohypophysis*, 157 (Butterworth Scientific Publications, London, Engl., 275 pp., 1957)
8. Sawyer, W. H., in *The Neurohypophysis*, 171 (Butterworth Scientific Publications, London, Engl., 275 pp., 1957)
9. Smith, W. H., *Am. J. Med.*, **23**, 623 (1957)
10. Wesson, L. G., Jr., *Medicine*, **36**, 281 (1957)
11. Rhodin, J., *Am. J. Med.*, **24**, 661 (1958)
12. Brun, C., and Raaschou, F., *Am. J. Med.*, **24**, 676 (1958)
13. Bradley, S. E., and Wheeler, H. O., *Am. J. Med.*, **24**, 692 (1958)
14. Milne, M. D., Scribner, B. H., and Crawford, M. A., *Am. J. Med.*, **24**, 709 (1958)
15. Berliner, R. W., Levinsky, N. G., Davidson, D. G., and Eden, M., *Am. J. Med.*, **24**, 730 (1958)
16. Pitts, R. F., *Am. J. Med.*, **24**, 745 (1958)
17. Relman, A. S., and Schwartz, W. B., *Am. J. Med.*, **24**, 764 (1958)
18. Taggart, J. V., *Am. J. Med.*, **24**, 774 (1958)
19. Mudge, G. H., *Am. J. Med.*, **24**, 785 (1958)
20. Yamada, E., *J. Biophys. Biochem. Cytol.*, **1**, 551 (1955)

21. Hall, V., *Am. Heart J.*, **54**, 1 (1957)
22. Pak Poy, R. K. F., *Australian J. Exptl. Biol. Med. Sci.*, **35**, 583 (1957)
23. Pak Poy, R. K. F., *Australian J. Exptl. Biol. Med. Sci.*, **35**, 437 (1957)
24. Pak Poy, R. K. F., and Robertson, J. F., *J. Biophys. Biochem. Cytol.*, **3**, 183 (1957)
25. Mueller, C. B., *Am. Heart J.*, **55**, 304 (1958)
26. Pappenheimer, J. R., and Kinter, W. B., *Am. J. Physiol.*, **185**, 377 (1956)
27. Kinter, W. B., and Pappenheimer, J. R., *Am. J. Physiol.*, **185**, 399 (1956)
28. Waugh, W. H., *Circulation Research*, **6**, 363 (1956)
29. Thompson, D. D., Kavalier, F., Lozano, R., and Pitts, R. F., *Am. J. Physiol.*, **191**, 493 (1957)
30. Kessler, R. H., Heidenreich, O. P. A., and Pitts, R. F., *Am. J. Physiol.*, **191**, 501 (1957)
31. Waugh, W. H., *Circulation Research*, **6**, 107 (1958)
32. Waugh, W. H., and Hamilton, W. F., *Circulation Research*, **6**, 116 (1958)
33. Swann, H. G., Ormsby, A. A., Delashaw, J. B., and Tharp, W. W., *Proc. Soc. Exptl. Biol. Med.*, **97**, 517 (1958)
34. Goodyer, A. V. N., Mattie, L. R., and Chetrick, A., *Am. J. Physiol.*, **193**, 360 (1958)
35. Lilienfeld, L. S., Rose, J. C., and Porfido, F. A., *Circulation Research*, **5**, 64 (1957)
36. Ochswadt, B., *Arch. ges. Physiol.*, **265**, 112 (1957)
37. Brull, L., and Louis-Bar, D., *Arch. internat. physiol. et biochem.*, **65**, 470 (1957)
38. Hollander, W., Michelson, A. L., and Wilkins, R. W., *Circulation*, **16**, 246 (1957)
39. Kuschinsky, G., Jungblut, P., Vorherr, H., and Cullman, B., *Arch. exptl. Pathol. Pharmacol.*, **231**, 473 (1957)
40. Blatteis, C. M., and Horvath, S. M., *Am. J. Physiol.*, **192**, 353 (1958)
41. Hinshaw, L. B., and Bradley, G. M., *Am. J. Physiol.*, **189**, 329 (1957)
42. Blatteis, C. M., and Horvath, S. M., *Am. J. Physiol.*, **192**, 357 (1958)
43. Burch, G. E., and Philips, J. H., *Circulation Research*, **6**, 72 (1958)
44. Janssen, S., and Grupp, G., *Arch. exptl. Pathol. Pharmacol.*, **230**, 245 (1957)
45. His, E. L., *Am. J. Physiol.*, **192**, 191 (1958)
46. Engelhorn, R., *Arch. exptl. Pathol. Pharmacol.*, **231**, 219 (1957)
47. White, A. G., *Am. J. Physiol.*, **191**, 50 (1957)
48. Huang, K. C., King, N. P., and Genazzani, E., *Am. J. Physiol.*, **192**, 373 (1958)
49. Despopoulos, A., and Weissbach, H., *Am. J. Physiol.*, **189**, 548 (1957)
50. Deyrup, I. J., *J. Gen. Physiol.*, **41**, 49 (1957)
51. Cohen, J. J., Berglund, F., and Lotspeich, W. D., *Am. J. Physiol.*, **189**, 331 (1957)
52. Braun, W., Whittaker, V. P., and Lotspeich, W. D., *Am. J. Physiol.*, **190**, 563 (1957)
53. Whittaker, V. P., *Acta Physiol. Scand.*, **42**, Supp. 145, 136 (1957)
54. Jones, R. T., and Blake, W. D., *Am. J. Physiol.*, **193**, 371 (1958)
55. Jones, R. T., and Blake, W. D., *Am. J. Physiol.*, **193**, 375 (1958)
56. Lindell, S. E., and Schayer, R. W., *Brit. J. Pharmacol.*, **13**, 44 (1958)
57. Lindell, S. E., and Schayer, R. W., *Brit. J. Pharmacol.*, **13**, 52 (1958)
58. Lindell, S. E., and Schayer, R. W., *Brit. J. Pharmacol.*, **13**, 89 (1958)
59. Lindahl, K. M., and Sperber, I., *Acta Physiol. Scand.*, **42**, 166 (1958)
60. Kandel, A., Green, R. E., Volle, R. L., and Peters, L., *J. Pharmacol. Exptl. Therap.*, **122**, 327 (1958)

61. Stj6ren, E. J., *Am. J. Physiol.*, **192**, 387 (1958)
62. Herndon, R. F., and Freeman, S., *Am. J. Physiol.*, **192**, 369 (1958)
63. Waddell, W. J., and Butler, T. C., *J. Clin. Invest.*, **36**, 1217 (1957)
64. Waddell, W. J., and Butler, T. C., *Proc. Soc. Exptl. Biol. Med.*, **96**, 563 (1957)
65. Doolan, P. D., Harper, H. A., Hutchin, M. E., and Alpen, E. L., *Am. J. Med.*, **23**, 416 (1957)
66. Halmi, N. S., King, L. T., Widney, R. R., Hass, A. C., and Stuelke, R. G., *Am. J. Physiol.*, **193**, 379 (1958)
67. Berman, L. B., and Houck, J. C., *Proc. Soc. Exptl. Biol. Med.*, **97**, 175 (1958)
68. McGeachin, R. L., and Hargan, L. A., *Proc. Soc. Exptl. Biol. Med.*, **95**, 341 (1957)
69. Hoenig, V., *Lancet*, **I**, 506 (1958)
70. Huffman, E. R., Hlad, C. J., Jr., Whipple, N. E., and Elrick, H., *J. Clin. Invest.*, **37**, 369 (1958)
71. Gutman, A. B., and Yü, T. F., *Bull. N. Y. Acad. Med.*, **34**, 287 (1958)
72. Gutman, A. B., and Yü, T. F., *Am. J. Med.*, **23**, 600 (1957)
73. Cullen, J. H., LeVine, M., and Fiore, J. M., *Am. J. Med.*, **23**, 587 (1957)
74. Yu, T. F., Berger, L., Stone, D. J., Wolf, J., and Gutman, A. B., *Proc. Soc. Exptl. Biol. Med.*, **96**, 264 (1957)
75. Yu, T. F., Sirota, J. H., Berger, L., Halpern, M., and Gutman, A. B., *Proc. Soc. Exptl. Biol. Med.*, **96**, 809 (1957)
76. Reed, E. B., Feichtmeier, T. V., and Willett, F. M., *New Engl. J. Med.*, **258**, 894 (1958)
77. Czaczkes, W. J., Ullmann, T. D., and Sadowsky, E., *J. Lab. Clin. Med.*, **51**, 224 (1958)
78. Verney, E. B., *Lancet*, **II**, 1237, 1295 (1957)
79. Dicker, S. E., and Nunn, J., *J. Physiol. (London)*, **141**, 332 (1958)
80. Campagna, M. J., Dodge, H. W., Jr., and Clark, E. C., *Am. J. Physiol.*, **191**, 59 (1957)
81. Taylor, N. B. G., Hunter, J., and Johnson, W. H., *Can. J. Biochem. and Physiol.*, **35**, 1017 (1957)
82. Vial, S. V., Croxatto, H., and Barnafi, L., *J. Appl. Physiol.*, **11**, 227 (1957)
83. Walker, J. M., in *The Neurohypophysis*, 221 (Butterworth Scientific Publications, London, Engl., 271 pp., 1957)
84. Papper, S., Saxon, L., Burg, M. B., Seifer, H. W., and Rosenbaum, J. D., *J. Lab. Clin. Med.*, **50**, 692 (1957)
85. Brunner, H., Kuschinsky, G., and Peters, G., *Arch. exptl. Pathol. Pharmacol.*, **233**, 1 (1958)
86. Brunner, H., Kuschinsky, G., and Peters, G., *Arch. exptl. Pathol. Pharmacol.*, **233**, 19 (1958)
87. Smith, H. W., *Principles of Renal Physiology* (Oxford University Press, New York, N. Y., 237 pp., 1956)
88. Wirz, H., *Helv. Physiol. et Pharmacol. Acta*, **14**, 353 (1956)
89. Wirz, H., in *The Neurohypophysis*, 157 (Butterworth Scientific Publications, London, Engl., 275 pp., 1957)
90. Wirz, H., Hargitay, B., and Kuhn, W., *Helv. Physiol. et Pharmacol. Acta*, **9**, 196 (1951)
91. Wirz, H., *Helv. Physiol. et Pharmacol. Acta*, **11**, 20 (1953)
92. Hollander, W., Jr., Williams, T. F., Fordham, C. C., III, and Welt, L. G., *J. Clin. Invest.*, **36**, 1059 (1957)

93. Sawyer, W. H., *Am. J. Physiol.*, **189**, 564 (1957)
94. Orloff, J., Wagner, H. N., Jr., and Davidson, D. G., *J. Clin. Invest.*, **37**, 458 (1958)
95. Berliner, R. W., and Davidson, D. G., *J. Clin. Invest.*, **36**, 1416 (1957)
96. Kleeman, C. R., Maxwell, M. H., and Rockney, R., *Proc. Soc. Exptl. Biol. Med.*, **96**, 189 (1957)
97. Epstein, F. H., Kleeman, C. R., and Henriks, A., *J. Clin. Invest.*, **36**, 629 (1957)
98. Grande, F., Taylor, H. L., Anderson, J. T., Buskirk, E., and Keys, A., *J. Appl. Physiol.*, **12**, 202 (1958)
99. Dicker, S. E., *J. Physiol. (London)*, **139**, 108 (1957)
100. DeWardener, H. E., and Herxheimer, A., *J. Physiol. (London)*, **139**, 42 (1957)
101. Epstein, F. H., Kleeman, C. R., Pursel, S., and Hendriks, A., *J. Clin. Invest.*, **36**, 635 (1957)
102. Meroney, W. H., Rubini, M. E., and Blythe, W. B., *Ann. Internal Med.*, **48**, 562 (1958)
103. Blackmore, K. E., and Schnieden, H., *Brit. J. Pharmacol.*, **12**, 279 (1957)
104. Richter, H. S., *Proc. Soc. Exptl. Biol. Med.*, **97**, 141 (1958)
105. Oliver, J., MacDowell, M., Welt, L. G., Holliday, M. A., Hollander, W., Jr., Winters, R. W., Williams, T. F., and Segar, W. E., *J. Exptl. Med.*, **106**, 563 (1957)
106. Hollander, W., Jr., Winters, R. W., Williams, T. F., Bradley, J., Oliver, J., and Welt, L. G., *Am. J. Physiol.*, **189**, 557 (1957)
107. Raisz, L. G., McNeely, W. F., Saxon, L., and Rosenbaum, J. D., *J. Clin. Invest.*, **36**, 767 (1957)
108. Croxatto, H., and Zamorano, B., *Acta Physiol. Latinoam.*, **7**, 33 (1957)
109. Rosenbaum, J. D., Papper, S., Cohen, H. W., and McLean, R., *J. Clin. Invest.*, **36**, 1202 (1957)
110. Schwartz, W. B., Bennett, W., Curelop, S., and Bartter, F. C., *Am. J. Med.*, **23**, 529 (1957)
111. Schlegel, J. U., and Stone, H., *Am. J. Physiol.*, **190**, 287 (1957)
112. Buchborn, E., *Endocrinology*, **61**, 375 (1957)
113. Robinson, K. W., and Macfarlane, W. V., *Endocrinology*, **60**, 679 (1957)
114. Dicker, S. E., and Nunn, J., *J. Physiol. (London)*, **138**, 11 (1957)
115. Heller, H., and Zaidi, S. M. A., *Brit. J. Pharmacol.*, **12**, 284 (1957)
116. Ginsburg, M., *J. Endocrinology*, **16**, 217 (1957)
117. Dettelbach, H. R., *Am. J. Physiol.*, **192**, 379 (1958)
118. Gottschalk, C. W., and Mylle, M., *Am. J. Physiol.*, **189**, 323 (1957)
119. Brunner, H., Kuschinsky, G., Peters, G., and Vorherr, H., *Arch. exptl. Pathol. Pharmacol.*, **233**, 57 (1957)
120. Ayres, P. J., Gould, R. P., Simpson, S. A., and Tait, J. F., *Biochem. J.*, **63**, 19 (1956)
121. Ayres, P. J., Hechter, O., Sauda, N., Simpson, S. A., and Tait, J. F., *Biochem. J.*, **65**, 22 (1956)
122. Giroud, C. J. P., Stachenko, J., and Venning, E. H., *Proc. Soc. Exptl. Biol. Med.*, **92**, 154 (1956)
123. Giroud, C. J. P., Saffran, M., Schally, A. V., Stachenko, J., and Venning, E. H., *Proc. Soc. Exptl. Biol. Med.*, **92**, 855 (1956)
124. Travis, R. H., and Farrell, G. L., *Federation Proc.*, **17**, 324 (1958)
125. Singer, B., and Stack-Dunne, M. P., *Nature*, **174**, 790 (1954)
126. Singer, B., and Stack-Dunne, M. P., *J. Endocrinol.*, **12**, 130 (1955)

127. Sweat, M. L., and Farrell, G. L., *Proc. Soc. Exptl. Biol. Med.*, **87**, 615 (1954)
128. Farrell, G. L., Rauschkolb, E. W., and Royce, P. C., *Am. J. Physiol.*, **182**, 269 (1955)
129. Rauschkolb, E. W., Farrell, G. L., and Koletsky, S., *Am. J. Physiol.*, **184**, 55 (1956)
130. Luetscher, J. A., Jr., and Axelrad, B. J., *J. Clin. Endocrinol. and Metabolism*, **14**, 1086 (1954)
131. Llaurodo, J. G., *Metabolism, Clin. and Exptl.*, **6**, 556 (1957)
132. Lieberman, A. H., and Luetscher, J. A., Jr., *Arch. Internal. Med.*, **100**, 774 (1957)
133. Rauschkolb, E. W., and Farrell, G. L., *Endocrinology*, **59**, 526 (1956)
134. Rauschkolb, E. W., Yatsu, F. M., and Farrell, G. L., *Proc. Endocrine Soc., 39th Meeting*, 32 (1957)
135. Mulrow, P. J., Lieberman, A. H., Shmagranoff, G. L., and Luetscher, J. A., Jr., *Clin. Res. Proc.*, **5**, 69 (1957)
136. Orti, E., Ralli, E. P., Laken, B., and Dumm, M. E., *Am. J. Physiol.*, **191**, 323 (1957)
137. Liddle, G. W., Duncan, L. E., Jr., and Bartter, F. C., *Am. J. Med.*, **21**, 380 (1956)
138. Muller, A. F., Riondel, A. M., and Manning, E. L., *Lancet*, **II**, 1021 (1956)
139. Venning, E. H., Dyrenfurth, I., Giroud, C. J. P., and Beck, J. C., *Can. Med. Assoc. J.*, **77**, 773 (1957)
140. Venning, E. H., Dyrenfurth, I., and Beck, J. C., *J. Clin. Endocrinol. and Metabolism*, **16**, 1541 (1956)
141. Venning, E. H., Carballeira, A., and Dyrenfurth, I., *J. Clin. Endocrinol. and Metabolism*, **14**, 784 (1954)
142. Luetscher, J. A., Jr., and Johnson, B. B., *J. Clin. Invest.*, **33**, 1441 (1954)
143. Axelrad, B. J., Johnson, B. B., and Luetscher, J. A., Jr., *J. Clin. Endocrinol. and Metabolism*, **14**, 783 (1954)
144. Cope, C. L., and Garcia-Llaurodo, J., *Brit. Med. J.*, **I**, 1290 (1954)
145. Farrell, G. L., Fleming, R. B., Rauschkolb, E. W., Yatsu, F. M., McCally, M., and Anderson, C. H., *Endocrinology*, **62**, 506 (1958)
146. Selkurt, E. E., *Physiol. Revs.*, **34**, 287 (1954)
147. Gaunt, R., and Chart, J. J., *Symposium on Homeostatic Mechanisms* (Brookhaven Natl. Lab., Brookhaven, L. I., N. Y., June, 1957)
148. Gordon, G. L., and Goldner, F., *Am. J. Med.*, **23**, 543 (1957)
149. Daily, W. J. R., and Ganong, W. F., *Endocrinology*, **62**, 442 (1958)
150. Newman, A. E., Redgate, E. S., Yatsu, F. M., and Farrell, G. L., *Federation Proc.*, **17**, 117 (1958)
151. Ayres, P. J., Garrod, O., Simpson, S. A., Tait, J. F., Walker, G., and Pearlman, W. H., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 309 (1957)
152. Muller, A. F., and Mach, R. S., *Réunion d'endocrinol.*, **4**, 25 (1957)
153. Johnson, B. B., Lieberman, A. H., and Mulrow, P. J., *J. Clin. Invest.*, **36**, 757 (1957)
154. Hernando, L., Crabbé, J., Ross, E. J., Reddy, W. J., Renold, A. E., Nelson, D. H., and Thorn, G. W., *Metabolism Clin. and Exptl.*, **6**, 518 (1957)
155. Bartter, F. C., Liddle, G. W., Duncan, L. E., Jr., Barber, J. K., and Delea, C., *J. Clin. Invest.*, **35**, 1306 (1956)
156. Muller, A. F., Riondel, A. M., and Manning, E. L., *Helv. Med. Acta*, **23**, 610 (1956)
157. Vesin, P., and Catlan, R., *Semaine hôp.*, **3**, 67 (1957)

158. Laragh, J. H., and Stoerck, H. C., *J. Clin. Invest.*, **36**, 383 (1957)
159. Muller, A. F., Manning, E. L., and Riondel, A. M., *Am. Assoc. Clin. Invest.*, **37**, 918 (1958)
160. Grossman, J., *Arch. Internal Med.*, **99**, 93 (1957)
161. Fine, D., Meiselas, L. E., and Auerbach, T., *J. Clin. Invest.*, **37**, 232 (1958)
162. Davis, J. O., Pechet, M. M., Ball, W. C., Jr., and Goodkind, M. J., *J. Clin. Invest.*, **36**, 689 (1957)
163. Ball, W. C., Jr., and Davis, J. O., *Am. J. Physiol.*, **191**, 339 (1957)
164. Davis, J. O., Bahn, R. C., Goodkind, M. J., and Ball, W. C., Jr., *Am. J. Physiol.*, **191**, 329 (1957)
165. Davis, J. A., and Ball, W. C., Jr., *Am. J. Physiol.*, **192**, 538 (1958)
166. Dyrenfurth, I., Stacey, C. H., Beck, J. C., and Venning, E. H., *Metabolism, Clin. and Exptl.*, **6**, 544 (1957)
167. Driscoll, T. E., Maultsby, M. M., Farrell, G. L., and Berne, R. M., *Am. J. Physiol.*, **191**, 140 (1957)
168. McCally, M., Anderson, C. H., and Farrell, G. L., *Proc. Endocrine Soc. 40th Meeting*, 119 (1958)
169. Henry, J. P., and Pearce, J. W., *J. Physiol. (London)*, **131**, 572 (1956)
170. Bartter, F. C., *Proc. Roy. Soc. Med.*, **51**, 201 (1958)
171. Noble, S., *Proc. Soc. Exptl. Biol. Med.*, **95**, 679 (1957)
172. Coxon, R. V., Dupré, J., and Robinson, R. J., *Quart. J. Exptl. Physiol.*, **43**, 86 (1958)
173. Taymor, R. C., and Friedberg, C. K., *J. Appl. Physiol.*, **11**, 125 (1957)
174. Balint, P., Fekete, A., Hajou, A. C., Kiss, E., and Pethes, G., *Acta Med. Scand.*, **158**, 261 (1957)
175. Judson, W. E., Hollander, W., and Wilkins, R., *J. Lab. Clin. Med.*, **49**, 672 (1957)
176. Thomas, S., *J. Physiol. (London)*, **139**, 337 (1957)
177. Wagner, J. N., Jr., *J. Clin. Invest.*, **36**, 1319 (1957)
178. Bricker, N. S., Straffon, R. A., Mahoney, E. P., and Merrill, J. P., *J. Clin. Invest.*, **37**, 185 (1958)
179. Fischer, A., Takacs, L., and Varga, S., *Z. ges. exptl. Med.*, **129**, 33 (1957)
180. Bricker, N. S., and Gregory, L., Jr., *J. Lab. Clin. Med.*, **51**, 546 (1958)
181. Love, A. H. G., Roddie, R. A., Rosensweig, J., and Shanks, R. G., *Clin. Sci.*, **16**, 281 (1957)
182. Lauson, H. D., and Thompson, D. D., *Am. J. Physiol.*, **192**, 198 (1958)
183. Blake, W. D., *Am. J. Physiol.*, **191**, 393 (1957)
184. Langston, J. B., and Guyton, A. C., *Am. J. Physiol.*, **192**, 131 (1958)
185. Lathem, W., Marks, P. A., Roof, B. S., and Bradley, S. E., *J. Lab. Clin. Med.*, **50**, 588 (1957)
186. Nicholson, T. F., *Can. J. Biochem. and Physiol.*, **35**, 641 (1957)
187. West, C. D., and Bayless, R. K., *Am. J. Physiol.*, **191**, 512 (1957)
188. Beck, R. N., *Clin. Sci.*, **17**, 37 (1958)
189. Nicholson, T. F., *Can. J. Biochem. and Physiol.*, **35**, 419 (1957)
190. Brodsky, W. A., Miley, J. F., Kaim, J. T., and Shah, N. P., *Am. J. Physiol.*, **193**, 108 (1958)
191. Richterich, R., Goldstein, L., and Dearborn, E. H., *Am. J. Physiol.*, **192**, 392 (1958)
192. Williamson, B. J., and Freeman, S., *Am. J. Physiol.*, **191**, 384 (1957)

193. Anderson, H. M., and Laragh, H., *J. Clin. Invest.*, **37**, 323 (1958)
194. Foulkes, E. C., Hanenson, I. B., and Miller, B. F., *Proc. Soc. Exptl. Biol. Med.*, **95**, 272 (1957)
195. Laron, Z., Crawford, J. D., and Klein, R., *Proc. Soc. Exptl. Biol. Med.*, **96**, 649 (1957)
196. Levitt, M. F., Halpern, M. H., Polimeros, D. P., Sweet, A. Y., and Gribetz, D., *J. Clin. Invest.*, **37**, 294 (1958)
197. Kyle, L. H., Schaaf, M., and Canary, J. J., *Am. J. Med.*, **24**, 240 (1958)
198. McGown, M. G., *Clin. Sci.*, **16**, 297 (1957)
199. Berglund, F., and Forster, R. P., *J. Gen. Physiol.*, **41**, 429 (1958)
200. Levinsky, N. G., and Davison, D. G., *Am. J. Physiol.*, **191**, 530 (1957)
201. Schmidt-Nielsen, K., Jørgensen, C. B., and Osaki, H., *Am. J. Physiol.*, **193**, 101 (1958)
202. Kessler, R. H., Lozano, R., and Pitts, R. F., *J. Clin. Invest.*, **36**, 656 (1957)
203. Greif, R. L., and Jacobs, G. S., *Am. J. Physiol.*, **192**, 599 (1958)
204. Heinemann, H. O., and Becker, E. L., *J. Appl. Physiol.*, **12**, 51 (1958)
205. Surtshin, A., and Parelman, A. G., *Proc. Soc. Exptl. Biol. Med.*, **95**, 628 (1957)
206. Surtshin, A., *Am. J. Physiol.*, **190**, 271 (1957)
207. Surtshin, A., and Yagi, K., *Am. J. Physiol.*, **192**, 405 (1958)
208. Beyer, K. H., Jr., Baer, J. E., Russo, H. F., and Noll, R., *Science*, **127**, 146 (1958)
209. Goodkind, M. J., Harvey, R. M., and Richards, D. W., *Am. J. Med. Sci.*, **235**, 164 (1958)
210. Bayliss, R. I. S., Marrack, D., Pirkis, J., Rees, J. R., and Zilva, J., *Lancet*, **I**, 120 (1958)
211. Laragh, J. H., Heinemann, H. O., and Demartini, F. E., *J. Am. Med. Assoc.*, **166**, 145 (1958)
212. Rochelle, J. B., Moyer, J. H., and Ford, R. V., *Am. J. Med. Sci.*, **235**, 168 (1958)
213. Seldin, D. W., Rector, F. C., Jr., and Teng, H. C., *Am. J. Physiol.*, **189**, 551 (1957)
214. Capeci, N. E., Kruesi, O. R., Weaver, D. C., and Hilton, J. G., *Am. J. Physiol.*, **191**, 55 (1957)
215. Freeman, S., and Jacobsen, A. B., *Am. J. Physiol.*, **191**, 388 (1957)
216. Olewine, D. A., and Perlmutter, J. H., *Am. J. Physiol.*, **192**, 592 (1958)
217. Coulson, R. A., Hernandez, T., and Beebe, J. L., *Proc. Soc. Exptl. Biol. Med.*, **96**, 606 (1957)
218. Leiter, L., *Bull. N. Y. Acad. Med.*, **34**, 143 (1958)
219. Friedberg, C. K., *Circulation*, **16**, 437 (1957)
220. Goldberger, E., *Acta Med. Scand.*, **157**, 417 (1957)
221. Derow, H. A., *New Eng. J. Med.*, **258**, 77, 124 (1958)
222. Moore, F. D., *New Eng. J. Med.*, **258**, 325, 427 (1958)
223. Mudge, G. H., *Bull. N. Y. Acad. Med.*, **34**, 152 (1958)
224. Hollander, W., and Judson, W. E., *J. Clin. Invest.*, **36**, 1460 (1957)
225. Cottier, P. T., Weller, J. M., and Hoobler, S. W., *Circulation*, **17**, 750 (1958)
226. Hollander, W., and Judson, W. E., *Circulation*, **17**, 576 (1958)
227. Genest, J., Koiw, E., Nowaczynski, W., and Leboeuf, G., *Proc. Soc. Exptl. Biol. Med.*, **97**, 676 (1958)
228. Genest, J., Lemieux, G., Davignon, A., Koiw, E., Nowaczynski, W., and Steyermark, P., *J. Clin. Invest.*, **35**, 706 (1956)

229. Conn, J. W., *Circulation*, **17**, 743 (1958)
230. Dustan, H. P., and Masson, G. M. C., *Circulation*, **17**, 765 (1958)
231. del Greco, F., Masson, G. M. C., and Corcoran, A. C., *Am. J. Physiol.*, **191**, 525 (1957)
232. Albert, D. G., Morita, Y., and Iseri, L. T., *Circulation*, **17**, 761 (1958)
233. Heinemann, H. O., and Cheung, M. W., *J. Lab. Clin. Med.*, **49**, 923 (1957)
234. Bearn, A. G., Yu, T. F., Gutman, A. B., *J. Clin. Invest.*, **36**, 1107 (1957)
235. Dorhout Mees, E. J., *Acta Med. Scand.*, **157**, 199 (1957)
236. Bricker, N. S., Shwayri, E. I., Reardan, J. B., Kellogg, D., Merrill, J. P., and Holmes, J. H., *Am. J. Med.*, **23**, 554 (1957)
237. Linke, A., and Dowlatabadi, H., *Klin. Wochschr.*, **36**, 78 (1958)
238. Remenchik, A. P., Schoenberger, J. A., and Dyniewicz, J. M., *Am. J. Med. Sci.*, **235**, 189 (1958)
239. Dobson, A., and Phillipson, A. T., *J. Physiol. (London)*, **140**, 94 (1958)
240. Heinz, E., and Durbin, R. P., *J. Gen. Physiol.*, **41**, 101 (1957)
241. Curran, P. F., and Solomon, A. K., *J. Gen. Physiol.*, **41**, 143 (1957)
242. Solomon, S., *J. Cellular Comp. Physiol.*, **49**, 351 (1957)
243. Huf, E. G., and Wills, J., *J. Gen. Physiol.*, **36**, 473 (1953)
244. Ussing, H. H., and Zerahn, K., *Acta Physiol. Scand.*, **23**, 110 (1951)
245. Linderholm, H., *Acta Physiol. Scand.*, **27**, Suppl. 97 (1952)
246. Bentley, P. J., *J. Endocrinol.*, **16**, 126 (1957)
247. Huf, E. G., Doss, N. S., and Wills, J. P., *J. Gen. Physiol.*, **41**, 397 (1957)
248. Garby, L., Risholm, L., Thoren, O., and Ulfendahl, H., *Urol. Intern.*, **5**, 207 (1957)
249. Hlad, C. J., Jr., Nelson, R. E., Holmes, J. H., and Stoll, F., *Am. J. Physiol.*, **184**, 406 (1956)
250. Vivion, C. G., Hlad, C. J., and Eiseman, B., *J. Urol.*, **79**, 471 (1958)
251. Leaf, A., Anderson, J., and Page, L. B., *J. Gen. Physiol.*, **41**, 657 (1958)
252. Weyrauch, H. M., and Roland, S. I., *J. Urol.*, **79**, 255 (1958)
253. Persky, L., Levey, S., and Abbott, W. E., *J. Urol.*, **79**, 463 (1958)
254. Gagnon, J. A., and Clarke, R. W., *Am. J. Physiol.*, **190**, 117 (1957)
255. Alexander, D. P., Nixon, D. A., Widdas, W. F., and Wohlzogen, F. X., *J. Physiol. (London)*, **140**, 14 (1958)
256. Levine, J., and Levine, A. D., *Am. J. Physiol.*, **193**, 123 (1958)
257. Dignam, W. J., Titus, P., and Assali, N. S., *Proc. Soc. Exptl. Biol. Med.*, **97**, 512 (1958)
258. Dupré, J., and Coxon, R. V., *Quart. J. Exptl. Physiol.*, **43**, 74 (1958)
259. Koike, T. I., and Kellogg, R. H., *Am. J. Physiol.*, **191**, 45 (1957)
260. Andersson, B., and Wyrwicka, W., *Acta Physiol. Scand.*, **41**, 194 (1957)

RESPIRATION^{1,2}

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REGULATION OF RESPIRATION

RESPIRATORY CENTERS

Central neural control of respiration has been the subject of considerable study and active debate. Wang *et al.* (232) have shown that the isolated medulla can maintain rhythmic respiration without the influence of spinal or vagal afferents but that the medullary respiratory centers are not the pace makers for normal respiration. They believe that in normal respiration the rhythm originates in the pontile apneustic center, with periodic modulation by inhibitory impulses both from the pulmonary stretch receptors in the vagus and from the pneumotaxic center in the rostral pons. Ngai & Wang (173) have explored the entire lower brain stem region by electrical stimulation and circumscribed electrolytic destruction and have localized the pneumotaxic center in the dorsolateral reticular formation of the isthmus of the pons. Electrical stimulation of this region resulted in respiratory acceleration with some reduction of amplitude, and vagotomy did not consistently alter the response. Bilateral destruction of this area in the vagotomized animal resulted in apneustic respiration. The apneustic center is probably localized in the lateral reticular formation of the middle pons and extends medially toward the caudal portion. The medullary inspiratory center is localized in the ventral reticular formation rostral to and at the level of the obex. This confirms the previous finding of Pitts *et al.* (182). However, the expiratory center is localized further caudally in a circumscribed area in the dorsal portion of the medulla, 1 to 3 mm. caudal to the obex. Electrical recording of the area showed spontaneous discharges synchronous with expiratory movements [Haber *et al.* (98)].

Redgate & Gellhorn (186) reported that lesions in the lateral hypothalamus result in an immediate reduction in rate or depth of respiration, or both, in lightly anesthetized cats. This appears to indicate that the pathways from the hypothalamic panting mechanism to the pontile pneumotaxic center have been cut off and that the former may exert a facilitating action on the lower centers.

¹ The survey of the literature pertaining to this review was concluded in May, 1958.

² The symbols used are completely explained in *Federation Proceedings* 9, 602 (1950). A large capital signifies a gas volume; small capitals refer to the gas phase; a dot above a large capital indicates a time element. Among the abbreviations used are the following: FRC (functional residual capacity), RV (residual volume), V_D (respiratory dead space), PVR (pulmonary vascular resistance), MBC (maximum breathing capacity), A (alveolar), a (arterial), v (venous), P (pressure).

Careful pulmonary ventilatory studies in pontile and medullary cats have been made by Ngai (172). Respiratory acidosis is observed regularly in animals exhibiting the apneustic respiration. Ten per cent CO_2 inhalation increases the amplitude of the inspiratory spasm and accelerates the apneustic cycle. This response persists after carotid sinus denervation. In medullary animals, respiratory acidosis also occurs if the respiration is slow or shallow. Their oxygen consumption is maintained at approximately the normal rate until shortly before death.

Tang *et al.* (224) have presented evidence of a close relationship between the vasomotor and respiratory centers. The latter can affect the former directly or via ventilatory blood pressure fluctuation. The direct action can be prevented by inhibiting the respiratory center by hyperventilation, and the indirect action may be abolished by pneumothorax or by asphyxia.

Earlier studies have shown that a continuous infusion of either epinephrine or norepinephrine in man or in lightly anesthetized animals causes a transient stimulation of respiration. The transience of the hyperpnea results from hypocapnia, because when the subjects were given a mixture of air containing 2 to 5 per cent CO_2 so that the alveolar CO_2 would remain high during the infusion of norepinephrine, a sustained increase of 20 to 50 per cent in pulmonary ventilation was obtained (11). The respiratory stimulation by epinephrine was carefully analyzed by Young (249). It appears that the epinephrine hyperpnea is independent of the integrity of the vagus, the spinal cord below the level of the eighth cervical vertebrae, and the entire visceral content. Neither is it definitely a direct effect on the respiratory center, because intra-arterial injection of the amine does not influence respiration.

VAGUS NERVES

Several papers deal with effects of vagotomy. Oberholzer & Schlegel (175) observed that unanesthetized or barbitalized guinea pigs succumbed 30 to 180 min. after vagotomy. They suggest that the autonomous activity of the guinea pig's respiratory center depends on the vagal facilitating impulses from the lungs. Honda *et al.* (112) observed that bilateral vagotomy brought about a marked increase in respiratory response to 2 to 6 per cent CO_2 in the inspired air. They believed that the vagal nerve depresses the excitability of the respiratory center to chemical stimuli. Zechman *et al.* (251) observed that even though the respiratory frequency was reduced when vagi were blocked by cooling, work per minute was increased by virtue of the large increase in the work required for each cycle. Clemenson (42) reported that the rapid shallow breathing in normal rabbits on exposure to high explosive shock waves was completely absent after vagotomy. The afferents are believed to be in the pulmonary branches of the vagus nerves. Wyss (246) recorded phrenic potentials in rabbits inhaling O_2 - CO_2 mixtures. The inspiratory synchronization that resulted from vagotomy led him to conclude that this inspiratory synchronization is a result of dyspneic activation as well as of release from vagal inhibition of inspiratory activity.

Leusen *et al.* (142) studied the CO_2 and O_2 concentration and the pH of the arterial blood in normal and debuffered dogs at rest and during exercise and observed no important differences in the latter animals. This result indicates that adaptation of the ventilation caused by the increased metabolism during exercise can be maintained in the absence of the arterial chemo- and pressoreceptors. Youmans & Schopp (248) observed Cheyne-Stokes respiration in two debuffered dogs and concluded that neither oxygen-lack drive from chemoreceptors nor reflex effects on breathing from rhythmic fluctuations in pressure in the sinoaortic zones are concerned invariably in the production of this abnormal respiratory pattern.

OTHER PERIPHERAL RECEPTORS

Dejours *et al.* (62) designed experiments on normal human subjects to ascertain whether or not specific ventilatory chemoreceptors were present in the legs. The evidence indicates that these are not present. The authors suggest two possible explanations for the ventilatory changes during leg exercise: modifications of the proprioceptive ventilatory stimulus by local chemical changes, or the existence of specific chemoreceptors central to the working muscles. They further suggest that the establishment of an equilibrium between the receptors and their chemical environment is a probable component of the eventual development of the steady state (61).

In the Union of Soviet Socialist Republics, Sergievski & Ivanov (201) made an interesting study concerning the patterns of respiratory response evoked by carbon dioxide in dogs with modified receptor systems. They found that in animals deprived of visual, auditory, and olfactory receptors, the sensitivity to carbon dioxide was reduced immediately following operation. After some time the sensitivity rose again, but the initial level was never regained. Furthermore, respiratory response to carbon dioxide could be enhanced by raising the cortical excitability, and the reduction of sensitivity after carotid sinuses denervation could be concealed by conditioned reflexes. They suggested that the normal level of carbon dioxide sensitivity of dogs depends upon the functional condition of the analyzing system including centers for analyzers of the cerebral cortex, rather than upon the "activating system of the reticular formation". In the reviewer's opinion these experimental findings do not contradict the concept that respiratory function is controlled from the reticular substance of the lower brain stem but rather indicate that sensory inflow from whatever source tends to reinforce the respiratory center's response to carbon dioxide. This important concept may lead to fruitful research into the physiology of the respiratory center.

EFFECT OF CARBON DIOXIDE AND OXYGEN

Landmesser, Cobb, and their co-workers studied the respiratory response to carbon dioxide "transients" in normal subjects and in patients under diethyl ether and cyclopropane anesthesia (45, 137). They found that in normal subjects the respiratory response in terms of alveolar ventilation

ratio (VaR) varies directly with the carbon dioxide stimulus in terms of P_{CO_2} of the internal jugular blood. Thus, this furnishes a more reliable estimate of respiratory response than similar measurements taken from arterial blood and end expiratory or inspiratory gas. Using the same technique, Cobb *et al.* (45) showed that neither diethyl ether nor cyclopropane produced definite change in the sensitivity of the respiratory center to carbon dioxide, as indicated by the similar slopes of the stimulus-response curves obtained, but the threshold of ventilatory response to carbon dioxide is lowered slightly by cyclopropane and considerably by diethyl ether. This observation tends to refute the common belief that cyclopropane is a potent respiratory depressant.

Pauli *et al.* (178) showed that the responsiveness of the respiratory center to carbon dioxide in patients with mitral valvular disease was diminished, while there was no evidence of carbon dioxide accumulation. The mechanisms remain to be elucidated.

Winters *et al.* (244) followed the changes of blood pH, plasma CO_2 content, and P_{aCO_2} in patients recovering from metabolic acidosis. These patients frequently pass through a phase at which blood pH is normal or alkaline but P_{aCO_2} is still depressed. The sustained hyperventilation that this suggests may be explained by an increased sensitivity of the respiratory center to carbon dioxide or H-ion, or both.

Yamamoto (247) reported that in anesthetized rats the carbon dioxide exchange is similar to those reported in man and dog. The homeostatic defense against internally produced carbon dioxide (electrically induced exercise) is good, while that against environmental carbon dioxide exchange is poor.

Loeschke (146) perfused the fourth ventricle of anesthetized, chemoreceptor-debuffered cats with bicarbonate buffer solution of various pH and carbon dioxide tensions. He found that a shifting of pH to acid with constant carbon dioxide tension brought about an increase in respiration, while shifting of pH to alkaline caused a decrease in respiration. Also, an increase of carbon dioxide tension with constant pH led to inhibition, and a decrease of carbon dioxide tension led to an increase in the respiration. Furthermore, his experimental results led him to conclude that the receptive substrate is superficially located. These receptors are sensitive to H-ions and are most probably located in the choroid plexus of the fourth ventricle.

The tonic hypoxemic stimulus of respiration, probably from the chemoreceptors, was again demonstrated in man by May (160). When subjects were switched from room air to 60:40 O_2/N_2 inhalation, the mean minute respiratory volume was reduced during the first two minutes, primarily owing to a decrease in V_T and secondarily to a decrease in respiratory rate. On the other hand, Baker & Hitchcock (7) found that in man 100 per cent oxygen inhalation was accompanied by an increase in ventilation volume, carbon dioxide output, and respiratory rate. They attributed these effects to a partial loss of the "dual function" of hemoglobin, the stimulation of respiratory center by increased carbon dioxide tension, and H-ion concentration.

Cormack, Cunningham & Gee (48) studied the effect of anoxia on respiration when P_{ACO_2} was maintained at or above normal level. A given degree of anoxia was found to cause a significantly greater respiratory response at a higher level of P_{ACO_2} in man. The relationship between P_{O_2} and the slope of the \dot{V} - P_{CO_2} line as shown by Nielsen and Smith was reinvestigated in detail by Lloyd *et al.* (145) and was confirmed. Furthermore, since the \dot{V} - P_{AO_2} relationship with several constant values of P_{ACO_2} has also been found to show positive interaction between the respiratory effects of the two gases, the hypothesis of Gray (94) that these effects are simply additive was therefore contradicted.

Hull (117) analyzed the respiratory response of anesthetized dogs to progressive anemic hypoxia produced by hemodilution or by breathing dilute carbon monoxide in air. The tidal volume underwent progressive diminution. The breathing rate increased while the alveolar ventilation remained normal or slightly below normal. Respiratory failure occurred when the blood oxygen capacity was reduced to 20 to 30 per cent of normal.

DRUG EFFECTS ON RESPIRATORY CENTER

The possible role of acetylcholine as a central synaptic neurohumoral transmitter in respiratory control has been suggested by numerous previous experiments, but conclusive evidence of this relationship is still lacking. Metz (164) studied this problem in dogs by attempting to correlate the level of acetylcholinesterase activity in the brain and the degree of reflex hyperpnea upon stimulation of Hering's nerve after the administration of various doses of an anticholinesterase. From analysis of the acetylcholinesterase activity of representative brain areas vitally concerned with respiration, Metz demonstrated that following an initial marked potentiation of the respiratory reflex, maximum at an acetylcholinesterase level 84 to 88 per cent of control, there is a progressive decline in the reflex potentiation which parallels the fall in acetylcholinesterase, and respiratory failure occurs when the enzyme activity falls to 8 to 11 per cent of control. These findings are compatible with a cholinergic factor in the central control of breathing. The action of this cholinergic factor at the central synapses was shown indirectly by the antagonistic action of morphine and an anticholinesterase in rats, mice, or guinea pigs by Schaumann (198), who suggested that the acetylcholine output is reduced by morphine, and this reduced amount is stabilized by the anticholinesterase. However, the author failed to show the respiratory stimulating effect of acetylcholine as a result of anticholinesterase injection.

Williams *et al.* (242) demonstrated that in dogs 2,4-dinitrophenol (DNP) produced hyperventilation and respiratory alkalosis preceding any significant rise in body temperature. Although small doses of dinitrophenol stimulate respiration through chemoreceptor activation, a direct central effect with larger doses was shown to occur in chemoreceptor-denervated animals. It is possible that this agent increased the sensitivity of the respiratory center to carbon dioxide or H-ion concentration in a manner similar to the effect of

salicylates. An interesting phenomenon was an unexplained increase in "buffer base" during respiratory alkalosis. A similar observation in the reverse direction was made by Holaday *et al.* (111) in that the "buffer base" tended to decrease during acute respiratory acidosis, the degree of decrease correlating with the severity of the respiratory component of acid-base disturbance.

Samet *et al.* (197) re-evaluated the effect of salicylates upon the ventilatory response to carbon dioxide in patients with pulmonary emphysema and hypocapnia. In contrast to the results reported earlier by Wégria (234), these authors failed to observe any significant increase in ventilation in response to inhalation of various concentrations of carbon dioxide when blood salicylate levels were between 11 to 30 mg. per cent. Neither did salicylates alter the arterial blood oxygen saturation and carbon dioxide tension when the subjects were breathing room air. These results suggested that salicylates do not increase the sensitivity of the respiratory center to carbon dioxide. This seems to agree with the concept of Graham & Parker (93), who showed in 1948 that the hyperventilation from salicylates is caused by reflex stimulation through vagal afferents and is independent of the blood acid-base balance. The exact mechanism, however, appears to be in need of further elucidation.

Barak, Beber, & Jacobi (10), who investigated the effect of acetazoleamide (Diamox) and ammonium chloride on acid-base balance in pulmonary emphysema, found that both of these agents have the same end result, i.e., lowering of arterial blood carbon dioxide tension. Since symptomatic relief found with acetazoleamide was not observed with ammonium chloride, they questioned that the salutary effect of the former in patients with pulmonary emphysema can be attributed to the lowering of alveolar carbon dioxide tension alone. Zuniga-Caro & Orrego-Puelma (252) attempted to elucidate this problem in patients with respiratory acidosis. Their results point to the hypothesis that in hypercapnia modifications of the buffer properties in the blood and tissue produce a depressing action on the respiratory center. With added intra-extracellular ionic modifications that lead to alterations of the membrane potential, the lack of response of the respiratory center to carbon dioxide or other stimuli might be explained. Acetazoleamide was assumed to act on the blood and tissue buffer systems, progressively re-establishing the sensitivity of the respiratory center and thus permitting pulmonary elimination of carbon dioxide. This accords with the results obtained by Galdston & Geeler (89) who studied the effects of acetazoleamide and aminophylline on ventilation and acid-base balance and on respiratory response to carbon dioxide in pulmonary emphysema. These authors reported that the respiratory center's sensitivity to carbon dioxide was not increased with these drugs even when there was a significant reduction in the levels of arterial carbon dioxide tension, plasma bicarbonate, and arterial pH. However, prior reduction in arterial carbon dioxide tension and plasma bicarbonate induced by acetazoleamide may account for the enhanced response to amino-

phylline as observed in some of their subjects. Similar results were obtained by Taquini *et al.* (225).

Kase & Borison (126) studied the central depressant action of hemicholinium in the cat. By means of electrical stimulation and transection of the lower brain stem, they analyzed the effect of hemicholinium on the central respiratory mechanism. Their findings led to the conclusion that the respiratory depressant action of this agent is caused mainly by its effect on the regulatory mechanism in the brain stem rather than by depression of the spinal cord. Based upon the concept of organization of central respiratory mechanism advanced by Brodie & Borison (29), they concluded that hemicholinium does not depress the "pacemaker" function but depresses the brain stem respiratory function in the following order: "modulator", "oscillator", and "integrator". Although larger doses of hemicholinium can cause neuromuscular blockade, this central depression takes precedence over its peripheral action.

HYPOVENTILATION

In a review by Woolmer (245) factors conducive to respiratory inadequacy were discussed. Methods to assess the adequacy of ventilation were enumerated and the management of respiratory inadequacy was elucidated. Fishman *et al.* (81) have directed attention to the syndrome of hypoventilation, characterized by hypoxemia, hypocapnia, and respiratory acidosis. In addition to disruption of normal balance between alveolar ventilation and perfusion and alveolar-capillary block, which are the physiologic manifestations of diverse pulmonary lesions, a third cause for the production of hypoventilation has been recognized. This occurs in patients with normal lungs but with poor performance of the chest bellows. The concept of "chest bellows" has been broadened to include not only the thoracic cage but also the diaphragm, the abdominal panniculus, and the abdominal content. Coates *et al.* (44) discussed the problem of hypoventilation syndrome and added still another cause for its production: the primary depression of the respiratory center by drugs, anesthesia, or disease. Administration of 100 per cent oxygen resulted in reduction of ventilation in all four of the patients they studied, and in symptoms of confusion and stupor in three of the four. Froeb *et al.* (88) reported that respiratory acidosis can be induced by oxygen breathing only in those patients who had maximum breathing capacity (MBC) and timed vital capacities below 40 to 50 per cent of their predicted values. The incidence rose markedly as patients with the most severe impairment were tested.

Efron & Kent (74) reported a case of chronic respiratory acidosis, probably of central origin, caused by encephalitis. Repeated cardiopulmonary studies failed to disclose any significant disease in the cardiopulmonary system, but the respiratory center's response to inhalation of carbon dioxide and salicylates was found to be abolished. An unusual electroencephalographic pattern in the response to hyperventilation was discovered and was

related to blood carbon dioxide content. It is interesting to note that this patient was able to drive the respiratory mechanism from higher voluntary centers, thus indicating that the pathways from upper brain stem to medulla were intact.

A series of reports dealt with the hypoventilation syndrome of obesity (77, 120, 144). Marked obesity was found to be associated with alveolar hypoventilation, arterial hypoxemia, hypercapnia, and secondary polycythemia in the absence of primary cardiopulmonary disease. Patients with this syndrome tend to show somnolence, periodic breathing, and eventually symptoms and signs of right-sided congestive heart failure. It was suggested that the mechanical effect of obesity in increasing the work of breathing is the primary factor in the genesis of alveolar hypoventilation.

The relationship between ventilation and the changes in P_{aCO_2} and arterial O_2 saturation was studied by Earle *et al.* (69). By reducing the alveolar ventilation in the dog in a stepwise fashion and simultaneously increasing the P_{IO_2} just enough to maintain the arterial O_2 saturation at 85 per cent, these authors found a reciprocal relation between effective \dot{V}_A and P_{aCO_2} , and also between \dot{V}_A and minimal P_{IO_2} required to maintain the arterial O_2 saturation at 85 per cent.

RESPIRATORY ADAPTATION

FETAL AND NEONATAL RESPIRATION

Newborn infants react to hypoxia quite differently from older children and adults. Cross *et al.* (50) measured the oxygen consumption, CO_2 production, minute volume, and respiratory quotient in newborn infants breathing 15 per cent oxygen and found that they respond to this mild anoxia by lowering their energy production and decreasing metabolic activity. These adaptations finally lead to a fall in body temperature. They concluded that: (a) lack of shivering should not be cited as evidence of immaturity of temperature-regulating mechanism since it is beneficial not to shiver if a fall in metabolism is a defense against anoxia; and (b) low body temperature may represent part of the adaptive process against anoxia rather than lack of control. Behrle & Smull (18) compared the somatic responses of very young (under 24 hr. of age) and older infants to mild hypoxia (10 to 12 per cent oxygen), and Miller (169) studied the relationship between hypoxia, apnea, and bradypnea in small, premature infants. These authors suggested that hypoxia, instead of acting as a stimulus to respiration as it does in older infants and adults, tends to depress the respiratory activity of newly born and premature infants. The chemoreflex function elicited by a fall in arterial oxygen tension is definitely lacking. In their cardiac and angiocardigraphic studies James & Rowe (121) found that infants with proven patent ductus arteriosus reacted poorly to hypoxia, their arterial oxygen saturation falling precipitously. Since the same authors (194) found that high pulmonary arterial pressure was present in normal infants under two weeks of age, it is reasonable to assume that persistence of the fetal type of circulation may very well be an important factor in accounting for the

poor adjustment to hypoxia of very young infants. The fetus can usually stand a relatively severe degree of hypoxia. Paff & Boucek (176) reported that chick embryo heart was able to withstand a gaseous environment of 4 per cent oxygen, and total anoxia in the presence of 4 per cent CO_2 for a considerable length of time.

A method was described for directly measuring ventilation and gas uptake in infants, and it was shown that alveolar ventilation, oxygen uptake, and diffusing capacity for carbon monoxide increase with their body weight (212). Gaseous metabolism of the newborn was studied by Cross *et al.* (49), using a body plethysmograph. A closer correlation was found between the minute volume of respiration and metabolism than between the minute volume and weight. A significantly higher serum bilirubin concentration in premature infants with high respiratory rate in the early hours of life was reported by Miller & Reed (168). This correlation, however, is poorly understood.

Respiratory distress is by far the most common single cause of death in the newborn. Its etiology, pathological physiology, and therapy were discussed in several publications (28, 65, 76, 92, 115, 187).

ABNORMAL BAROMETRIC PRESSURE

In a detailed review of problems of underwater swimming and diving, Taylor (226) gave an excellent account of the physiological processes involved in respiration of gases under pressure. The oxygen cost of forced breathing while submerged was found by Bartlett *et al.* (13) to be approximately twice that measured "in air". This was probably owing to greater mechanical work involved during underwater breathing, largely in overcoming inertia.

In the process of acclimatization to high altitudes, man must adapt to a lower alveolar carbon dioxide pressure to permit a higher oxygen pressure, but the nature of this adaptation remains unclear. This problem was well explored by Kellogg *et al.* (128). The respiratory stimulation produced by graded carbon dioxide inhalation was studied in man at sea level and during acclimatization to an altitude of 12,470 ft. The curve relating respiratory minute volume to inspired or alveolar carbon dioxide shifted to the left by 8 to 11 mm. Hg of P_{ICO_2} , while the slope and shape of the curve did not change significantly. This indicates primarily a change in the respiratory regulatory mechanism's threshold to the stimulus of carbon dioxide and is quite different from the respiratory response observed in acute hypoxia. These authors explain this response as a direct consequence of hyperventilation initiated by hypoxic stimulation of chemoreceptors. Alteration of mental performance in chronic anoxia was observed in members of a Mount Everest expedition party (95). The deterioration of mental performance of jet pilots was reported to be caused by hyperventilation in addition to hypoxia (9). An increase in venous blood lactic acid concentration was observed during hypoxia in simulated high altitude in human subjects, the increase depending on the degree and duration of hypoxia (104). The magnitude of increase

was poorly correlated to the functional disability. In animal experiments, post-mortem brain lactic acid concentration was found to be increased following altitude exposure (228), hypocapnia as well as hypoxia being necessary to cause this increase (20).

THERMAL STIMULI

During acute exposure to cold the respiratory minute volume, oxygen consumption, and respiratory quotient showed significant increase from exposure and shivering (211).

The effect of a rise in body temperature (1 to 3°C.) on the respiratory response to carbon dioxide was studied by Cunningham & O'Riordan (51). They found that the sensitivity to carbon dioxide was increased twofold, and at normal P_{aCO_2} the ventilation was increased by 3 to 16 L./min.

In hypothermia, respiratory rate and oxygen consumption fell exponentially with temperature (23). Severinghaus *et al.* (203) determined the physiological dead space and the arterial to alveolar carbon dioxide difference in dogs and man during hypothermia. They found that hypothermia did not result in block of carbon dioxide elimination, as was suggested by previous investigators. The influence of blood temperature alone on pulmonary circulation was shown in a carefully controlled study by Galletti & Salisbury (90). Dog lungs were perfused separately from the systemic circulation with blood at different temperatures, and the pulmonary hemodynamics were observed. The pronounced change of pulmonary vascular resistance (PVR) caused by cooling or heating was attributed to pulmonary vasomotion.

MECHANICS OF BREATHING

COMPLIANCE OF LUNG AND THORAX

Marshall (152) measured the lung volumes, compliance, and nonelastic resistance in 50 young normal subjects at rest. He attempted to correlate the lung compliance with FRC, RV, IC, VC, body surface area, and height. While there is a significant correlation between lung compliance and all these parameters, the best correlation is with functional residual capacity (FRC): compliance (l./cm. H_2O) = $0.05 \times FRC$ (liters). Butler *et al.* (35) studied the lung compliance of 33 normal subjects with various air flow rates throughout the vital capacity. They found a curvilinear S-shaped pressure-volume relationship in both static records (taken with very low airflow rate so as to approach static conditions) and spiral records (taken with tidal breath or increased depth of breath). The static records of pressure and volume during expiration showed a smaller transpulmonary pressure than the records of inspiration at corresponding volumes. It was suggested that the variations in the ventilated lung volume are dependent on the overcoming of surface tension forces. Frank *et al.* (86) investigated the mechanical behavior of the lungs in healthy elderly subjects and compared the findings with those in younger subjects. They found that while there was no significant difference in static compliance between elderly and younger subjects, in the former

the volume-pressure curve of the lung shifted to the left and the pulmonary flow-resistance was significantly higher. These changes in the physical properties of the lungs were similar in kind to those found in emphysema, though much smaller in degree.

Foster *et al.* (84) studied the lung compliance in ten normal subjects anesthetized and paralyzed with succinylcholine. Using the method of Dornhorst and Leathert, they observed that the lung compliance was not significantly different from normal values. Their results differ from those of Howell & Peckett (114) who found that the lung compliance decreased during anesthesia and muscular paralysis. However, the value of total thoracic compliance obtained by Foster *et al.* agrees well with that reported earlier by Nims *et al.* (174). The effect of curare on the elastic properties of chest and lung was studied by Massion (155) in anesthetized dogs. Using a method which measured chest cage and lung elastances separately, he found that administration of curare increased the total chest elastance by 48.1 per cent, the lungs contributing 42.2 per cent and the chest cage 5.9 per cent. He suggested that histamine release with resultant bronchoconstriction, as well as increase of surface tension in the alveoli caused by transudation, are possible factors responsible for the change in elastance.

Pulmonary vascular engorgement is known to reduce lung compliance, and Pryor *et al.* (183) demonstrated this in normal subjects following rapid infusion of 1000 ml. of human serum albumin in saline. These authors also showed that in patients with mitral stenosis, exercise caused a substantial increase in pulmonary vascular pressure and a considerable reduction of lung compliance except in those cases where the compliance was already low at rest. Cherniack *et al.* (41) measured the elastic and viscous resistance in orthopneic patients with congestive heart failure and found a marked increase in viscous resistance in supine position. The major part of this increased resistance occurred at the end of expiration and beginning of inspiration, when the lung volumes were low. As a result, the work of breathing was increased by 25 per cent. In these patients the orthopnea was tentatively attributed to the interference with the patency of the respiratory passages at low level of lung inflation. In contrast to chronic congestive heart failure or pulmonary hypertension, elevation of the left atrial pressure to 30 to 40 cm. H₂O or changes of pulmonary blood flow from 0 to 450 cc./kg. body wt./min. did not significantly alter the volume-pressure curve of the lung in dogs (25). Sharp *et al.* (204) described the ventilatory mechanics in eight patients with frank pulmonary edema, and in seven of the eight following recovery. They found that the compliance was very low, averaging 0.037 ± 0.0056 (S.E.) l./cm. H₂O during the pulmonary edema, and 0.089 ± 0.019 (S.E.) l./cm. H₂O following recovery. The inspiratory resistance was markedly increased, averaging 9.9 ± 0.83 (S.E.) cm. H₂O/l./sec. during pulmonary edema, and on recovery 5.3 ± 1.17 cm. H₂O/l./sec. They suggested that the presence of bubbles in alveoli might have a marked effect on compliance.

The elastic resistance of the rabbit lung was investigated by Bernstein

(19), Bucher (31, 32), and Kautsky (127). Bernstein (19) explored the non-linear component of the elastic pressure-volume relationship and found that there were both an "unadapted" and an "adapted" curve for the lung and thorax of a living rabbit. He explained the presence of these two curves by the difference in the number of functioning alveoli at the beginning and at the end of the period of inflation. It should be pointed out that the "unadapted" curve is quite different from the sigmoid curve reported for man, and there was no reference to the phenomenon of adaptation having been observed in the human lung. Bucher (31, 32) and Kautsky (127) measured the elastic resistance of lungs of rabbits and other small laboratory animals. The elastic resistance of the lung during spontaneous respiration was characterized by the factor $dp/\Delta V$, where $dp = (P_{alv} - P_{pi})$ inspiration - $(P_{alv} = P_{pi})$ - expiration, and $\Delta V = V_T/FRC$. Various alterations of respiratory activity induced by carbon dioxide inhalation, increase or decrease of the alveolar pressure, and decrease of FRC by pneumothorax were found not to change this factor. Clements *et al.* (43) attempted to clarify the dependence of static pressure-volume characteristics of the lung on internal surface force through some theoretical considerations based on changes of surface tension in the lung mucosa.

WORK OF BREATHING

The measurement of oxygen cost of breathing is a potentially valuable clinical tool but is not a usual pulmonary function test mainly because of difficulties in determining oxygen consumption by ordinary means. Bartlett *et al.* (14), who had previously reported the measurement of oxygen cost of breathing at low ventilatory volumes, now described three procedures for measuring the oxygen cost of breathing using the Collins respirometer or any similar metabolimeter. Data on oxygen cost of breathing over the entire ventilatory range were presented. The qualified use of vital capacity as an index of respiratory muscle function was discussed by Dail & Affeldt (53). Respiratory work was shown to be minimal with low respiratory frequencies (15 per min.) in man (138). Under high frequencies, as respirations are short and fast, dead space ventilation becomes important and resistance caused by viscosity and turbulence is increased. Zechman *et al.* (251) showed that in dogs cooling of the vagi increased the cost of breathing, even with low respiratory frequency. This author stated that the regulation of depth and frequency of breathing by the respiratory center is probably less economical when afferent discharges from pulmonary receptors are blocked.

RESISTANCE

Pulmonary functions were studied with the addition of variable resistance to the airway. The reduction in the timed vital capacity varied proportionally with the resistances imposed on tracheal air flow (100). This test gives a quantitative value which can be used to determine the degree of breathing obstruction in pulmonary efficiency tests. Maximal breathing capacity was determined with expiratory and inspiratory resistances (single and com-

bined) at various breathing rates (15). A given resistance in the inspiratory duct reduces the maximal breathing capacity much more than it does in the expiratory duct. A fourfold increase in the expiratory airway resistance lowers the maximal breathing capacity very little, so that special low resistance testing equipment does not seem to be necessary for the normal subject. The relatively unimportant role of expiratory muscles in pulmonary ventilation was also shown by Campbell *et al.* (36, 37), who used an electromyograph to measure the response of the abdominal muscles to increased expiratory resistances in normal subjects and found no significant abdominal muscle activity even when resistance pressure of over 10 cm. H₂O was imposed with high ventilation rates. The effect of respiratory impedances on pulmonary ventilation in the dog was studied by Hall & Zechman (101). When the tracheal airflow was impeded sufficiently to produce pronounced changes in inspiratory and expiratory pressures, breathing rate would increase and tidal volume decrease inversely with the pressure differences.

The extent to which an animal can constrict its trachea and bronchi apparently correlates with the development of peribronchial contractile muscles. The dog is known to have rich bundles of muscles in the tracheobronchial tree. Spontaneous, rhythmic contractions of trachea were recorded in anesthetized dogs by Loofbourrow *et al.* (147), who also studied various factors that induced strong contractions. In anticholinesterase poisoning the dog attained relative increase in lung-airway resistance four or more times greater than the monkey, which showed much less peribronchial smooth muscle anatomically (122).

ALTERATION OF PHYSICAL PROPERTIES OF THE LUNG BY PULMONARY DISEASES

Konar *et al.* (129) measured the intrapleural pressure in normal subjects and found that it is consistently about 2 cm. of H₂O less negative on the left side than on the right. Variations of intrapleural pressure in patients with pleural effusions, bronchial asthma, emphysema, and ascites were also described in the same paper. With induction of a large pneumothorax in the dog, ventilation coefficient was immediately increased and remained elevated, FRC was decreased, and oxygen consumption was not affected until after several hours (208). There was an immediate onset of rapid and shallow respiration with an increase in the minute volume and a lowering of the arterial blood carbon dioxide content. Vagotomy abolished this response, indicating that it was mediated through the vagal afferents. Surgical resection of diseased lung segments in moderately severe cases of bronchiectasis with impairment of pulmonary functions was followed by a progressively increasing inflation of lungs and improvement of maximal breathing capacity. Thus the removal of diseased lung apparently did not further impair the lung function (125). Decrease of lung compliance was shown to occur with pulmonary engorgement from left heart failure or intravenous infusion of albumin solution, as discussed earlier (41, 183). The physical properties of the lungs in patients suffering with chronic pulmonary diseases

with or without chronic cor pulmonale and right heart failure were studied by Hammond (103). All patients with emphysema and chronic cor pulmonale showed an increased inspiratory and expiratory nonelastic resistance compared with normal. The compliance was found to be low in emphysematous patients, but its mean value in patients with right heart failure was even lower. Recovery from heart failure was accompanied by decrease in non-elastic resistance and an increase in compliance. However, Mendel & McIlroy (162) found that the periodic breathing observed in some patients with pulmonary congestion is not caused by the waxing and waning pulmonary congestion nor any variation of the mechanical properties of the lung, but perhaps by periodic fluctuations of respiratory center activity. Michelson & Lowell (166) demonstrated that in patients with bronchial asthma the increase in respiratory symptoms induced by postural changes is not caused by the change in vital capacity and compartmental lung volume.

The alteration in many properties of the lungs that occurs in pulmonary emphysema was the subject of a critical review by Hugh-Jones (116). Other publications concerning pulmonary emphysema dealt with decrease in the intrapleural negative pressure and lung elasticity (103, 129), disturbances of ventilation (156), gas metabolism (157), and exaggeration of responses to Valsalva maneuver (167).

Respiratory insufficiency characterized by a decrease in MBC and IC was reported as a symptom of cerebellar ataxia by Hormia (113). The causal relationship between the disturbance of coordination and that of respiratory muscle activity needs further study. Alteration of pulmonary function in paraplegics was shown to depend on the location of the lesion (109).

EXERCISE

The respiratory functions during muscular work were examined by Ghiringhelli & Bosisio (91), using 15 healthy female subjects exercising on the Handle electroergometer. They found that pulmonary ventilation and oxygen consumption increased proportionally with muscular work until a work performance of 50 watts, corresponding to a caloric consumption of 4.00 cal./kg./hr. was reached. In a series of 171 male and 56 female tuberculous patients, definitely low physical working capacity was demonstrated (52), using the methods and standards of Wahlund and Sjostrand. This paper also reported a statistically significant correlation between VC as well as MCB and working capacity. The adaptation of ventilation to the increased metabolism during exercise is maintained in the absence of chemo- and pressor-receptors as shown by Leusen *et al.* (142) in the dog. The effect of passive motion of the legs on ventilation appears to be indisputable and has been discussed earlier (61, 62, 119).

MECHANICAL RESPIRATORS

In studying the effect of continuous pressure breathing on ventilation, Harboe & Lorentzen (105) found that in healthy subjects the nitrogen clearance pattern remained stable and that, in some, increased tidal volume gave more even ventilation.

Recent changes in the technique for using the tank respirator in the management of respiratory paralysis were described by Forbes of Melbourne, Australia (82). These involve the use of higher respiratory volume to one and one-half times greater or even more than those indicated by nomograms. This procedure maintains a smaller resting lung volume, prevents the occurrence of a relatively immobile distended thoracic cage with unopposed chest distension, and aids venous return. Walley (231) discussed the control of artificial respiration for poliomyelitis and stated that purely clinical assessment of the efficiency of positive-pressure respiration was unsatisfactory. He called for measurements of ventilatory volume by spirometry, estimation of end-tidal carbon dioxide concentration, blood gas studies, and chest radiography to supplement clinical observations.

Elam and co-workers (75) evaluated the effect of changes of lung-thorax compliance on the performance of a number of clinically used mechanical ventilators. They concluded that in the presence of wide variations and alterations of lung-thorax compliance, especially in anesthetized subjects, the pressure-limited ventilators will produce either hypo- or hyperventilation. On the contrary, certain volume-limited, pressure-variable ventilators can maintain the preset stroke volume within 10 to 20 per cent despite compliance variations of half to twofold. Measurement of airway pressure during the use of these volume-limited ventilators can also at least reveal the changes in lung-thorax compliance.

Two new respirators were described by Rochford *et al.* (191) and Greer & Donald (96). Both were designed for use during surgical anesthesia.

GAS EXCHANGE

MEASUREMENT OF GAS COMPOSITION AND TENSION

A new method for the measurement of blood oxygen tension using Clark's platinum electrode was described by Kreuzer *et al.* (133). The data obtained were compared with those obtained with the van Slyke as well as the Riley technique. Hackney *et al.* (99) reported a method for the estimation of P_{aCO_2} by determining the end-tidal P_{CO_2} and mixed venous P_{CO_2} , assuming that the arteriovenous P_{CO_2} difference is reasonably constant. They compared the estimated P_{aCO_2} values thus obtained with those obtained by direct measurement and found that the deviation was only 0.2 ± 2.9 (S.D.) mm. Hg. A basic formulation of methods for obtaining O_2 consumption data from measurement of P_{O_2} in open and closed circuit systems was made by Depocas & Hart (63). Accurate analysis of gases by refractive index measurement (73) and a method for microanalysis of CO_2 and O_2 in minute samples of gases were described (78).

VENTILATION AND PERFUSION

Respiratory dead space (V_D).—A few of the many factors known to alter the respiratory dead space were studied. Shepard *et al.* (206) measured the V_D by the single-breath method of Fowler and showed that it increases with increasing end-inspiratory lung volume but that this relationship was not linear. The relationship between V_D and the esophageal-mouth pressure

difference was very nearly linear. They suggested the likelihood that most of the distensibility of the dead space occurs in the small conduction airways. Severinghaus & Stupfel (202) reported that the anatomical V_D was reduced by hypoxia through bronchiolar constriction, but enlarged by epinephrine, oxygen, and increase in respiratory rate. These authors also discussed the occurrence of changes of alveolar dead space as a result of uneven distribution of pulmonary blood flow. In patients with moderate to severe pulmonary emphysema, the physiologic dead space was found to be 49 per cent of the V_T at rest (243). During exercise the ratio was 44 per cent. Again the physiologic dead space was taken as an index of effective alveolar perfusion. In the experiments of Bouhuys *et al.* (27), addition of an extra dead space of 260 and 480 cc. induced hyperventilation and an improved distribution of inspired air as measured by the open-circuit nitrogen washout method. However, the overall ventilatory efficiency was not significantly improved. A method to determine physiologic dead space indirectly from pulmonary ventilation and CO_2 production or O_2 consumption data obtained at different metabolic levels was reported by Margaria *et al.* (151). The data are valid (standard error less than 10 per cent) up to moderate levels of pulmonary ventilation.

Functional residual capacity (FRC) and residual volume (RV).—The measurement of FRC by the open-circuit nitrogen method of Darling, Cournand, and Richards was compared with that by open-circuit helium washout method (110). In normal subjects the agreement between the two methods was good, but in patients with emphysema a significantly larger mean value for FRC was obtained with the latter method. This discrepancy appears to result from the inability of the alveolar air to represent adequately the mean intrapulmonary gas concentration by the nitrogen method when there are severe defects in intrapulmonary gas mixing. When nitrogen was substituted for helium in the washout procedure, there was no significant difference in the value of FRC. A simple helium closed-circuit method for the measurement of RV was described by Motley (170), who tested it in 100 cases of all types of cardiopulmonary disturbances with marked variations in the absolute RV and total lung capacity. The measurement was compared with that obtained from the open-circuit nitrogen method and found to be accurate. The open-circuit nitrogen method was modified by Cohen (46) to include continuous monitoring of the expired nitrogen with a nitrogen meter for the detection of uneven alveolar ventilation. Carey *et al.* designed a portable carbon monoxide uptake apparatus for the estimation of FRC and pulmonary diffusion (39). Values of FRC obtained with this apparatus are claimed to be reproducible for field survey work, but the absolute values must be accepted with some reserve (207).

Uneven ventilation and ventilation-perfusion variations.—West *et al.* (236) described a simple single-breath method for measuring the unevenness of gas-blood distribution, using a mass spectrometer for the simultaneous continuous analysis of gas sampled at the lips. Their calculations showed that poorly ventilated alveoli were also poorly perfused (237). Campbell *et al.* (38) showed that various abnormal patterns of ventilation-perfusion distribution

occur during artificial ventilation. There was a significant increase in dead space but little increase in A-a P_{O_2} gradient, implying overventilation of parts of the lung which have a small pulmonary blood flow. However, considerable individual differences were seen. The influence of posture on ventilation and perfusion in normal subjects is discussed in detail in a bronchospirometric study by Svanberg (216). Lung volumes are found to be larger in sitting than in supine position. Ventilatory efficiency, studied for the first time in regard to posture changes by Svanberg (216), is also shown to be better in sitting position. In normal lungs the distribution of blood flow (oxygen uptake) is the same in sitting and supine positions, but in the lateral position a larger oxygen uptake from the lowermost lung is demonstrated. However, Svanberg stated in summary that changes in posture imply no hyper- or hypoventilation of a lung in relation to its perfusion despite the large postural changes in volume and ventilation. A balance apparently is being maintained, resulting in unchanged composition of the alveolar air and unchanged arterial blood gas tension. On the other hand, Martin & Young (154), using the same method of study, showed that redistribution of pulmonary blood flow favoring the lower lobe in normal subjects in the erect position results in a higher alveolar volume to blood flow ratio in the upper lobe. Larger alveolar volume to blood flow variations were found in tuberculosis patients, most of whom have upper lobe involvement and reduced blood flow even in the supine position. From these observations it is clear that it could be a serious error to assume that P_{aCO_2} equals P_{ACO_2} .

BLOOD GAS DIFFUSION

Several reports in the literature describe modified or new techniques for measuring the alveolocapillary gas diffusion. Dubois & Marshall (68) measured the exchange of gases between the alveoli and pulmonary capillaries during the whole respiratory cycle by determining the rate of absorption of N_2O continuously, using an ingenious manometric respirometer. They concluded that the exchange of inert, soluble gas between alveoli and capillaries continues evenly during both inspiration and expiration and that the pulmonary capillary blood flow remains constant throughout the respiratory cycle. Pulmonary diffusing capacity can be calculated from carbon monoxide equilibration data (33), but the limitation of the method is that varying diffusion in different lung areas cannot be demonstrated. Scherrer & Bucher (199) applied the method of the metabograph of Fleisch together with analysis of arterial blood gases for clinical measurement of diffusion capacity of the lung. From their measurements the normal value for the diffusion capacity at rest is 15 $cm.^3/min./mm.$ Hg, and during work 40 to 80 $cm.^3/min./mm.$ Hg, depending on age and size of the subject. A decrease of diffusion capacity at rest to 4 to 8 and during work to 10 to 20 $cm.^3/min./mm.$ Hg is indicative of a massive loss of active respiratory surface. A lightweight, portable box-bag containing a mixture of 0.1 per cent CO and 12 per cent helium in respirable air of a known constant composition was described by Carey *et al.* (39) for field survey work. In normal subjects, values

of carbon monoxide uptake obtained with this apparatus agreed well with determinations made with more elaborate laboratory methods (207). The apparent pulmonary diffusing capacity for CO was measured by Forster *et al.* (83) at alveolar O_2 tensions from 40 to more than 600 mm. Hg with both steady state and breath-holding methods. With either method it was shown to be decreased with increasing oxygen tension, varying as much as fivefold over the entire range. The pulmonary diffusion capacity obtained with the steady state method is on the average 0.85 of that obtained by the breath-holding method. Jones *et al.* (123) studied the relationship between blood carboxyhemoglobin saturation and alveolar partial pressure of CO during breath holding, and devised a simple method for determining the saturation of COHb.

Roughton & Forster (193) derived an equation, $1/DM + 1/\theta V_C = 1/D_L$ which relates the measured diffusing capacity of the pulmonary membrane (DM), the rate of uptake of CO by the red cells per mm. Hg CO tension (θ), and the blood volume of the pulmonary capillary bed (V_C). By making measurements of D_L at different PA_{O_2} , this equation can be solved graphically for DM and V_C . They demonstrated that from this calculation V_C is about 75 ml., which is in agreement with the estimate of Roughton, and DM is about twice the value of D_L . This implies that resistance of red cells to the uptake of CO is of equal importance with that of the pulmonary membrane to the diffusion of gas across it. The ability of lung tissue to yield CO_2 was studied in the isolated, perfused rabbit lung by Stamm & Bucher (213). Carbon dioxide was found to be emitted quickly to the alveolar space on sudden stopping of perfusion fluid, and the portion of CO_2 arising from the lung tissue was much larger than that from the perfusion fluid.

In pulmonary diseases, with impairment in diffusion, the accuracy of all available methods in measuring pulmonary diffusing capacity is open to question. Although it is impossible to calibrate any method against a theoretical ideal figure, the general form of reported results in various lung diseases using different techniques gave, surprisingly enough, a similar range of results, as shown by Bates (16). This probably indicates that all available methods are influenced in the same direction by similar causes of error. In diseases with known uneven ventilation, such as pulmonary emphysema, measurement of pulmonary diffusing capacity with the single breath method is accurate enough, but estimation of the mean alveolar CO tension by the steady state method using end-tidal samples will be in serious error when taken at rest (16, 153). However, Bates pointed out that during exercise the D_{CO} calculated from the end-tidal sample agrees closely with that calculated from an assumed value of respiratory dead space even in the presence of unequal gas distribution in the lung (16). Marshall (153) described a bloodless modification of Filley's steady state method, using the value of mixed P_{vCO_2} instead of PA_{CO_2} . This method gave results which were close to those of the single breath D_L measured in the same subjects. Diffusion insufficiency for oxygen was observed in cases of pulmonary hypertension with restriction of pulmonary capillary bed, probably because of rapid capillary blood flow

resulting in inadequate alveolar-capillary contact time (148). In normal subjects, inflation of a tightly fitted pneumatic suit around the lower half of the body to a pressure of 75 mm. Hg produced an acute increase in pulmonary arterial and wedge pressures of 25 mm. of Hg, but no significant change in pulmonary diffusing capacity (143). This was in sharp contrast to the observation made during exercise, where the rise in pulmonary arterial pressure of 15 mm. of Hg was associated with a D_L increase of about 50 per cent. This indicates that the size of capillaries is not influenced by the lateral pressure across their walls. Rise in cardiac output and fall in PVR, induced by exercise but not associated with the inflation of a pneumatic suit, obviously account for the change in diffusion. Shepard (205) presented equations from which the relationship of O_2 saturation of blood leaving the pulmonary capillaries and mean alveolocapillary P_{O_2} gradient was integrated. From these relationships he concluded that when exercise tolerance is limited by the diffusing capacity, the limitation should occur abruptly at a critical level of O_2 consumption.

BRONCHOSPIROMETRY

To make a correct interpretation of the values obtained at bronchspirometry, various factors influencing these values must be taken into consideration. These factors are enumerated and discussed by Birath *et al.* in a review of more than 600 bronchspirometric studies (21). Bronchspirometry was used in an extensive study of the influence of posture on lung function, as reviewed in a previous paragraph (216). Viikari & Autio (230) carried out bronchspirometric investigations in tuberculous patients during exercise in sitting positions because this was thought to be in better accordance with physiological conditions. Autio (3) also developed a radioplanimetric method for evaluation of differential lung functions, whereby he calculated the contribution of each lung to the total volume change. The results were compared with those obtained with bronchspirometric method. Autio claimed that this method can be used in place of bronchspirometry in a large percentage of clinical cases and serves to select cases for bronchspirometric studies. Measurement of bronchspirometric tracings was facilitated with the use of a new transparent plastic slide rule and tables for direct conversion of millimeter measurement of spirographic tracings into actual volume and flow rate (130).

PULMONARY CIRCULATION

PRESSURE AND FLOW

A number of publications by Takezawa (219, 220, 221) dealt with the study of pulmonary circulation. Specifically he re-evaluated the accuracy of pulmonary arterial wedge pressure as a reflection of the left atrial pressure. The criteria for correct measurement of wedge pressure were enumerated and the usefulness of this determination in the early diagnosis of left heart failure was illustrated by some 21 cases. However, the validity of wedge pressure as an index of left atrial pressure was questioned by Murphy (171),

who compared these pressures in 12 patients with various cardiac diseases. He found that the wedge pressure paralleled the left atrial pressure only when the latter was within normal ranges, but failed to reflect it by significant values when the left atrial pressures were elevated.

The pressure-flow relationship in the dog lung was studied by Lategola (139). With acute shifting of blood flow through occlusion of various branches of pulmonary artery, the pulmonary capillary bed attained its maximum capacity when blood flow was increased 250 to 300 per cent, which is comparable to flow that has been reported in man during exercise. Similar studies were carried out by Harrison *et al.* (108) in dogs. These authors found that acute increase in right heart pressure upon pulmonary arterial occlusion (75 to 85 per cent reduction of functioning lung volume) tended to decrease after a number of years but that pulmonary pressure would rise sharply again when demand for increase in pulmonary blood flow was imposed on the animal. Rimini *et al.* (189) described a method to divert the blood flow from a lung or a lobe towards the remaining pulmonary parenchyma by applying positive pressure of about 25 cm. H₂O to the bronchus of the area to be deprived of blood. This circulatory exclusion produced by compression of pulmonary vessels by the increased alveolar pressure can be maintained long enough to permit estimation of the potential functional value of the remaining parenchyma, and is quite useful in evaluating pulmonary functions before lung resections. The presence of right-left shunts can be diagnosed, without recourse to blood samples, from the "saturation-tension" curve relating oxygen saturation to alveolar P_{O₂}, as reported by Perkins *et al.* (179).

The pulmonary circulation in the dog was studied by Weil *et al.* (235), using separate perfusion circuits for the systemic and the pulmonary circulations. These authors also investigated the influence of physiological variables on pulmonary arterial pressure with reference to pressure-flow relationships, changes in pulmonary ventilation, inhalation of CO₂, pulmonary embolism, and changes of systemic arterial pressure. The effect of exercise on pulmonary circulation in patients with left to right shunt was investigated by Swan, Marshall & Wood (217). The increased demand on the heart was apparently met with little additional stress on the already overburdened pulmonary circulation. However, Bruce & John (30) reported that the total pulmonary blood flow was increased by 21 per cent when the posture was shifted from supine to sitting, with further increase during exercise in the up-right position.

An interesting report on the collateral blood flow in dog lung was made by Salisbury *et al.* (196). They estimated that the anastomotic channels carry 0.5 to 1.0 per cent of the total arterial blood flow, and observed large variations of this collateral flow under a number of experimental conditions. Vidone & Leibow (229) demonstrated that when both the pulmonary artery and vein of a lung are ligated in the dog, the bronchial artery will expand and ultimately supply the lung by way of precapillary anastomoses with branches of pulmonary artery. With similar connections the pulmonary vein is drained through the enlarged bronchial vein. Sixteen months after

ligation, the volume of collateral blood flow in a 15 kg. dog can approximate 800 cc. per min. Abrams (1) suggested a method for detecting increase in pulmonary arterial pressure or flow (or both) by comparing radiographically the ratio of calibers of pulmonary and peripheral vessels.

PULMONARY VASCULAR RESISTANCE (PVR)

In a review by de Burgh Daly (55), active control of the lesser circulation by nervous, humoral, and gaseous stimuli was critically appraised. He quoted experimental conditions in which passive influences on the pulmonary vasculature were eliminated, and concluded that in the dog it is fair to assume: (a) the upper thoracic sympathetic outflow contains adrenergic vasoconstrictor fibers to the pulmonary vascular bed, and (b) the upper thoracic sympathetic outflow and the vagi contain fibers which on stimulation may lower the PVR by direct action or as a result of bronchial vascular effects. However, no experiments on human subjects so far reported fulfill all the criteria which are essential for the demonstration of an active neural control.

Rudolph & Paul reported that in dogs intrapulmonary infusion of serotonin caused marked pulmonary hypertension and increase in PVR (195). The serotonin constrictor effect on the pulmonary vasculature was also studied by Rose & Lazaro (192) in dogs whose systemic arterial circulation was regulated by a pump. Promethazine, LSD-25, and 2-brom derivatives were potent blocking agents of the serotonin effect. De Burgh Daly & Wright (56, 57, 60) found the PVR to be elevated, but to a lesser degree after administration of an anticholinesterase [sarin or tetraethylpyrophosphate (TEPP)]. These authors presented evidence that serotonin, sarin, or TEPP all act on the pulmonary vascular bed proper. The effectiveness of hexamethonium directly administered into the pulmonary artery in lowering the pulmonary arterial pressure of patients with pulmonary hypertension was demonstrated by Balchum *et al.* (8) and Yu *et al.* (250). Apart from the fact that hexamethonium lowered the PVR, these authors also pointed out that shifting of blood from the pulmonary to the systemic circulation via the existing varicose anastomoses may also play a part in the pulmonary pressure and flow changes. Active pulmonary vasodilatation after intrapulmonary infusion of acetylcholine in man was also reported by Fritts *et al.* (87). The acetylcholine effect was enhanced when pulmonary arterial pressure had been raised by hypoxia. Storstein and co-workers (215) reported that theophylline ethylenediamine (TED) had a dilator effect on pulmonary vascular bed in patients with various lung diseases, although intrapulmonary injection of this drug was not used in the study.

Pulmonary vasoconstriction induced by hypoxia was again demonstrated (4, 26, 190). Through ingenious experiments, Aviado *et al.* (4) showed that the reflex pulmonary vasoconstriction from anoxia was a part of the massive sympathetic discharge, resulting from strong chemoreceptor stimulation when the arterial oxygen saturation was reduced by about 10 per cent. In the denervated perfused lung, the effect of anoxia was vasodilatation which re-

quired a more severe anoxemia: a diminution of arterial oxygen saturation by at least 30 per cent. Lanari-Zubiaur & Hamilton (136) ventilated one lung of a dog with air or oxygen and the other with nitrogen and concluded that though the anoxic lung receives a significantly smaller blood flow, the physiological consequence of PVR increased by anoxic breathing is of doubtful significance. Peters (181) reported that in isolated perfused lungs, elevation of alveolar carbon dioxide tension caused a significant decrease in PVR. This finding differs from previous reports by other investigators. However, it is interesting to note that the systemic effect of hypercapnia was more or less completely excluded in the experimental program. It seems to be valid to assume from his data that vasodilatation is the direct local effect of carbon dioxide on the pulmonary vasculature.

The subject of pressoreceptors in the pulmonary vascular bed was again brought into focus, and the role of the distal pulmonary vascular bed was emphasized (66, 190). Indeed, the pulmonary veins have come to be considered more and more as an active part of the pulmonary vasculature capable of responding to various stimuli including hypoxia. Pulmonary venular constriction was also reported to be responsible for the increase in pulmonary arterial pressure in the case of *E. coli* endotoxin poisoning (134), and for the rise of left atrial pressure in bronchial asthma (222).

The effect of stimulation of the carotid sinus baroreceptors and of the carotid body chemoreceptors on pulmonary circulation in dogs and cats was investigated by de Burgh Daly *et al.* (2, 58, 59). Stimulation of the baroreceptors by raising the carotid sinus perfusion pressure caused an increase in PVR in perfused dog lung. These responses occurred independently of changes in bronchomotor tone, left auricular pressure, and the integrity of the sympathetic and vagal nerves. They suggested that the change in pulmonary vascular bed is secondary to the reflex fall in systemic blood pressure, giving rise to a redistribution of blood between the bronchial and pulmonary beds (58). However, baroreceptor stimulation in the cat resulted in a fall in pulmonary arterial pressure together with the systemic hypotension. Bilateral carotid occlusion or section of the carotid sinus nerve gave the opposite effect (2). Stimulation of the chemoreceptors in the dog by changing the perfusate from arterial to venous blood resulted in a decrease in PVR. This response is apparently a reflex mechanism mediated through the vagal cholinergic fibers, since it is abolished after section of the sinus nerves or vagal nerves (59).

The factors that can alter PVR in man were studied in a patient with mitral stenosis complicated by an anomalous pulmonary venous connection to the superior vena cava (141). It was found that the pressure gradient across the anomalously draining lung was roughly three times that across the lung draining into the left atrium, yet the blood flow through each was approximately the same. The decreased PVR of the lung draining to the left atrium, is, according to these authors, at least in part attributed to passive dilatation of the vessels by the high left atrial pressure. The effect of inflation of the lungs on PVR in rabbits was reinvestigated by Burton & Patel (34),

who also reinterpreted data obtained from the literature. These authors pointed out that PVR fell when the lungs were distended with negative pressure inflation. With positive pressure inflation, PVR rose or fell, depending on the degree of inflation. Increase of PVR in the cat during positive pressure respiration was reported by Barer & Nusser (12).

PULMONARY EDEMA

Drenckhahn (67) studied the pathogenesis of epinephrine-induced pulmonary edema in anesthetized guinea pigs and rabbits. He thought that the epinephrine edema might be explained by the increased pressure in the pulmonary circuit following a systemic arterial pressure rise. The normal linear relationship between the pulmonary venous pressure and the central venous pressure was upset by epinephrine. Singer, Laurent, and their co-workers (140, 209, 210) produced bilateral pulmonary edema in dogs by injecting starch granules into a lobar branch of one pulmonary artery. The pulmonary edema in the injected and the noninjected lungs was obviously induced by different mechanisms. These mechanisms were discussed on the basis of pressure measurement from various sites of the pulmonary circuits (210) and the effect of various drugs, given to the dogs prior to or together with the starch injection (140). Experimental pulmonary edema was also produced in dogs in which the denervated lungs were perfused through the pulmonary artery by a systemic arterial shunt (80), in male albino mice exposed to a threshold concentration of ozone (158), and in guinea pigs after bilateral cervical vagotomy (200). The last group of investigators believed that pulmonary edema following vagal section is caused not by sympathetic overactivity, but by a loss of sympathetic activity necessary to maintain normal smooth muscle tone in the bronchioles or pulmonary vessels. Halmagyi *et al.* (102) reported that lobeline is effective in preventing drug-induced edema of the lungs. These authors believed that the lobeline protective effect is mediated through adrenal cortex.

DRUGS AFFECTING RESPIRATION

NARCOTICS AND NARCOTIC ANTAGONISTS

Various investigators have suggested that the cardiovascular and respiratory effects of morphine result at least partly from stimulation of chemoreflex mechanisms. This prospect was explored by Gruhzit (97) in anesthetized dogs and cats using morphine, codeine, and two morphinan analogues. Injection of these agents into the right atrium or pulmonary artery caused brief periods of apnea within less than three seconds. This respiratory effect was abolished by bilateral vagotomy or bilateral vagal cooling and was not observed when the agents were injected into the left ventricle. These findings led this author to conclude that stimulation of pulmonary receptors was responsible for a portion of the respiratory effects of these agents. The two circulatory effects, hypotension and bradycardia, however, were not abolished by vagotomy and also occurred when the injection was made into the left ventricle. It was unfortunate that this study was done on anesthetized

preparations, as it has been shown (239) that chloroform in low concentration sensitizes the pulmonary stretch receptors and in high concentration paralyzes them. Although different types of receptors may be involved, the chloroform may very well alter the respiratory responses under investigation. A similar objection may be raised to thiopental as the anesthetic agent, since it can modify the central respiratory mechanism (218).

Eckenhoff *et al.* (72) found that doses of dihydrocodeine which produce only a slight respiratory depression, as measured by the V-end tidal PCO_2 -relationship, predispose to hypotension upon posture stress. The effect of morphine and atropine on respiration in man was re-evaluated in terms of $\dot{V}\text{-PACO}_2$ curves by Steinberg *et al.* (214). Morphine in doses of 10 mg. was found to be a respiratory depressant, but atropine (0.6 mg.), contrary to common belief, was not found to be a respiratory stimulant.

The use of narcotic antagonists has become increasingly popular. N-allylnormorphine (Nalline) and levallorphan (Lorfan) are used extensively in this country. Studies from Belgium (135) showed that N-d-camphosulfonyl-phenylmethylaminopropanol (Camphamedrine) has similar properties. The antimorphine action of one of the antagonists, 2,4-diamino-5-phenyl thiazole (Daptazol), was studied in the rabbit by an analysis of the EEG waves (163). The slow rhythm caused by morphine was found to be eliminated, indicating that its depressing effects on the activating system were antagonized. A biological competition of the two compounds obviously exists, and this was again demonstrated by Megirian *et al.* (161), who tried various combinations of dosage of levallorphan and meperidine in human. They found that, regardless of the total dose, levallorphan to meperidine at a ratio of 1:60 produced no respiratory depression and better sedation. Weakley & Bergner (233) showed that N-allylnormorphine potentiated the respiratory depression produced by barbiturates in dogs. However, in patients who had been given a narcotic before ether anesthesia, the use of a narcotic antagonist did not alter the respiratory response to endogenous CO_2 (159).

The search for new narcotics goes on, but with little satisfactory result. One of these was Anileridine, a new synthetic narcotic which is chemically related to meperidine. It was found to be a potent narcotic in human beings, but produced respiratory depression of the same magnitude as that caused by meperidine even though it caused little or no such depression in animals (40). The respiratory depressant effects of thiobarbiturates in man were studied (218). This depression was more pronounced when a narcotic was used in combination (70).

SYMPATHOMIMETIC DRUGS

Most of the available information on pulmonary effects of sympathomimetic amines has been limited to epinephrine and norepinephrine. Aviado & Schmidt (5) were the first to study in detail the effects of 25 sympathomimetics on pulmonary and systemic circulation in dogs, and they revealed nine patterns of action. The authors made special mention of a new compound, 45-50[β -hydroxy- β -(2,5-diethoxyphenyl) isopropylamine], which is a

pulmonary hypotensive, but systemic hypertensive drug. The suitability of this drug in the treatment of pulmonary hypertension accompanying heat injury to the lung was suggested. Active pulmonary vasomotor responses caused in the dog by epinephrine and norepinephrine as well as by acetylcholine, 5-hydroxytryptamine, histamine, and aminophylline were also studied and reported by Borst *et al.* (24). The pulmonary vasodilator effect of aminophylline was investigated by Quimby *et al.* (184), who reported that this effect, as well as the effect of 40 other xanthines on pulmonary circulation, was not a selective one.

The bronchodilator effects of six sympathomimetic amines were studied in dogs by direct injection into the bronchial artery (6). Again, this action was not selective, because when the agents were given intravenously the minimum effective dose for bronchodilatation always elicited systemic circulatory effects. In patients with clinical manifestations of bronchospasm, subcutaneous epinephrine was found to be superior to intravenous aminophylline (131). The "pneumodilating" effect of a new aerosol named Aerolone (a synergistic mixture of isopropylarterenol, cyclopentylamine, procaine, atropine, and propyleneglycol) was demonstrated in isolated lungs of guinea pigs (54). The use of isopropylarterenol to elicit the patient's maximal response of increase in forced expiratory volume was suggested for the estimation of extent of lung damage (118).

The bronchoconstrictor effect of histamine and related substances (2- β -aminoethyl pyridine, 3- β -aminoethyl-1,2,4-triazole and 1-methyl-4- β -aminoethyl imidazole) was studied in guinea pigs (238). A comparison of the influence of 5-hydroxytryptamine on respiration in guinea pigs with that of histamine showed that the effects of these two drugs can be differentiated pharmacologically, but did not elucidate the basis for the difference (85).

TRANQUILIZING DRUGS

Chlorpromazine was used in treating bronchial asthma. However, it was found to have no specific bronchodilator effect (165). A number of papers reported on the effect of tranquilizing drugs on respiration. Intramuscular administration of chlorpromazine (17) and promethazine (71) was shown to have no deleterious effect on respiration, but alveolar hypoventilation, irregularity of respiratory rhythm, and decreased ventilatory response to CO₂ were reported when the drug was given intravenously (188).

GENERAL

Williams (240, 241) reviewed the subject of pulmonary function and discussed the pathological processes and experimental procedures that could bring about the impairment of different functions. He and Harden *et al.* (106) stressed the importance to the proper application of therapy of classifying pulmonary disease according to the functional impairment. Assessment of cardiopulmonary functions before surgery was discussed by Kovach (132).

Recent advances in the technique of bronchography included the use of a new contrast medium, Visciodol, a lipoidal sulfanilamide suspension that

does not penetrate the alveoli (180, 185), and the use of the Metras catheter that can be placed selectively in different parts of the lung (150, 185). Bronchography was reported to aid the diagnosis of endobronchial tuberculosis (177). Lyons reported the usefulness of angiocardiology in the diagnosis of pulmonary vascular lesions (149). Blasi & Catena used the angiocardio-gram to study the position of the lesser circulation in the collapsed lung (22).

The correlation of the histopathological changes of the pulmonary vascular bed (arterial medial hypertrophy and increase in elastic tissue) and congenital malformation of the heart associated with pulmonary venous obstruction was shown by Ferencz & Wammann (79). Dust is deposited in the lung primarily at the alveoli that project from the wall of the respiratory bronchioles (223). Progressive pathological changes in the pulmonary vascular bed and parenchyma in the dog lung following heat injury by hot, dry air were described by Harkhurim & Vigalok (107).

The various new apparatuses described in the literature include different types of oxygenators for cardiopulmonary bypass (47, 64, 124), a low resistance respiratory valve with greatly reduced dead space (227), and several types of respirators (82, 96, 191).

LITERATURE CITED

1. Abrams, H. L., *Stanford Med. Bull.*, **14**, 97 (1957)
2. Agostoni, E., Chinnock, J. E., and de Burgh Daly, M., *J. Physiol. (London)*, **137**, 447 (1957)
3. Autio, V., *Acta Med. Scand.*, **158**, Suppl. 329, 1 (1957)
4. Aviado, D. M., Jr., Ling, J. S., and Schmidt, C. F., *Am. J. Physiol.*, **189**, 253 (1957)
5. Aviado, D. M., Jr., and Schmidt, C. F., *J. Pharmacol. Exptl. Therap.*, **120**, 512 (1957)
6. Aviado, D. M., Jr., Wnuck, A. L., and De Beer, E. J., *J. Pharmacol. Exptl. Therap.*, **122**, 406 (1958)
7. Baker, S. P., and Hitchcock, F. A., *J. Appl. Physiol.*, **10**, 363 (1957)
8. Balchum, O. J., Gensini, G., and Blount, S. G., *J. Lab. Clin. Med.*, **50**, 186 (1957)
9. Balke, B., Wells, J. G., and Clark, R. T., Jr., *J. Aviation Med.*, **28**, 241 (1957)
10. Barak, A. J., Beber, M., and Jacobi, H. P., *Am. J. Med. Sci.*, **234**, 71 (1957)
11. Barcroft, H., Basnayake, V., Celander, O., Cobbold, A. F., Cunningham, D. J. C., Jukes, M. G. M., and Young, I. M., *J. Physiol. (London)*, **137**, 365 (1957)
12. Barer, G. R., and Nusser, E., *J. Physiol. (London)*, **138**, 103 (1957)
13. Bartlett, R. G., Jr., Brubach, H. F., and Specht, H., *J. Appl. Physiol.*, **11**, 377 (1957)
14. Bartlett, R. G., Jr., Brubach, H. F., and Specht, H., *J. Appl. Physiol.*, **12**, 413 (1958)
15. Bartlett, R. G., Jr., and Specht, H., *J. Appl. Physiol.*, **11**, 79 (1957)
16. Bates, D. V., *J. Clin. Invest.*, **37**, 591 (1958)
17. Baum, G. L., Schotz, S. A., Gumpel, R. C., and Osgood, C., *Diseases of Chest.*, **32**, 574 (1957)
18. Behrle, F. C., and Smull, N. W., *Pediatrics*, **20**, 601 (1957)
19. Bernstein, L., *J. Physiol. (London)*, **138**, 473 (1957)

20. Biddulph, C., Van Fossan, D. D., Criscuolo, D., and Clark, R. T., Jr., *Am. J. Physiol.*, **193**, 345 (1958)
21. Birath, G., Stållberg-Stenhagen, S., and Swenson, E. W., *Am. Rev. Tuberc. Pulmonary Diseases*, **75**, 699 (1957)
22. Blasi, A., and Catena, E., *Poumon et le coeur*, **13**, 467 (1957)
23. Blatteis, C. M., and Horvath, S. M., *Am. J. Physiol.*, **192**, 357 (1958)
24. Borst, H. G., Berglund, E., and McGregor, M., *J. Clin. Invest.*, **35**, 669 (1957)
25. Borst, H. G., Berglund, E., Whittenberger, J. L., Mead, J., McGregor, M., and Collier, C., *J. Clin. Invest.*, **36**, 1708 (1957)
26. Borst, H. G., Whittenberger, J. L., Berglund, E., and McGregor, M., *Am. J. Physiol.*, **191**, 446 (1957)
27. Bouhuys, A., Jönsson, R., and Lundin, G., *Acta Physiol. Scand.*, **39**, 105 (1957)
28. Brand, I. K., *Conn. State Med. J.*, **22**, 88 (1958)
29. Brodie, D. A., and Borison, H. L., *Am. J. Physiol.*, **188**, 347 (1957)
30. Bruce, R. A., and John, G. G., *Circulation*, **16**, 776 (1957)
31. Bucher, K., *Helv. Physiol. et Pharmacol. Acta*, **15**, 315 (1957)
32. Bucher, K., *Helv. Physiol. et Pharmacol. Acta*, **15**, 328 (1957)
33. Burrows, B., and Harper, P. V., Jr., *J. Appl. Physiol.*, **12**, 283 (1958)
34. Burton, A. C., and Patel, D. J., *J. Appl. Physiol.*, **12**, 239 (1958)
35. Butler, J., White, H. C., and Arnott, W. M., *Clin. Sci.*, **16**, 709 (1957)
36. Campbell, E. J. M., *J. Physiol. (London)*, **136**, 556 (1957)
37. Campbell, E. J. M., Howell, J. B. L., and Peckett, B. W., *J. Physiol. (London)*, **136**, 563 (1957)
38. Campbell, E. J. M., Nunn, J. F., and Peckett, B. W., *Brit. J. Anaesthesia*, **30**, 166 (1958)
39. Carey, G. C. R., Phair, J. J., Shepard, R. J., and Thomson, M. L., *Arch. Ind. Health*, **16**, 225 (1957)
40. Chang, F. F. C., Safar, P., and Lasagna, L., *J. Pharmacol. Exptl. Therap.*, **122**, 370 (1958)
41. Cherniack, R. M., Cuddy, T. E., and Armstrong, J. B., *Circulation*, **15**, 859 (1957)
42. Clemedson, C. J., *Am. J. Physiol.*, **190**, 467 (1957)
43. Clements, J. A., Brown, E. S., and Johnson, R. P., *J. Appl. Physiol.*, **12**, 262 (1958)
44. Coates, E. O., Jr., Brinkman, G. L., and Noe, F. E., *Ann. Internal Med.*, **48**, 50 (1958)
45. Cobb, S., Converse, J. G., and Landmesser, C. M., *Anesthesiology*, **19**, 359 (1958)
46. Cohen, A. A., Hemingway, A., and Hemingway, C., *J. Clin. Invest.*, **37**, 306 (1958)
47. Cooley, D. A., Belmonte, B. A., Latson, J. R., and Pierce, J. F., *J. Thoracic Surg.*, **35**, 131 (1958)
48. Cormack, R. S., Cunningham, D. J. C., and Gee, J. B. L., *Quart. J. Exptl. Physiol.*, **42**, 303 (1957)
49. Cross, K. W., Tizard, J. P. M., and Trythall, D. A. H., *Acta Paediat.*, **46**, 265 (1957)
50. Cross, K. W., Tizard, J. P. M., and Trythall, D. A. H., *Acta Paediat.*, **47**, 217 (1958)
51. Cunningham, D. J. C., and O'Riordan, J. L. H., *Quart. J. Exptl. Physiol.*, **42**, 329 (1957)
52. Dahlström, G., *Acta Tuberc. Scand.*, **34**, 226 (1957)
53. Dail, C. W., and Affeldt, J. E., *Arch. Phys. Med. Rehabil.*, **38**, 383 (1957)

54. Dautrebande, L., Delaunois, A. L., and Heymans, C., *Acta Physiol. et Pharmacol. Neerl.*, **6**, 745 (1957)
55. de Burgh Daly, I., *Quart. J. Exptl. Physiol.*, **43**, 2 (1957)
56. de Burgh Daly, M., *Brit. J. Pharmacol.*, **12**, 504 (1957)
57. de Burgh Daly, M., *J. Physiol. (London)*, **139**, 250 (1957)
58. de Burgh Daly, I., and de Burgh Daly, M., *J. Physiol. (London)*, **137**, 427 (1957)
59. de Burgh Daly, I., and de Burgh Daly, M., *J. Physiol. (London)*, **137**, 436 (1957)
60. de Burgh Daly, M., and Wright, P. G., *J. Physiol. (London)*, **139**, 273 (1957)
61. Dejours, P., Mithoefer, J. C., and Labrousse, Y., *J. Appl. Physiol.*, **10**, 372 (1957)
62. Dejours, P., Mithoefer, J. C., and Raynaud, J., *J. Appl. Physiol.*, **10**, 367 (1957)
63. Depocas, F., and Hart, J. S., *J. Appl. Physiol.*, **10**, 388 (1957)
64. Diettert, G. A., Bercu, B. A., and Ferguson, T. B., *J. Thoracic Surg.*, **35**, 416 (1958)
65. Donald, I., *Brit. J. Anaesthesia*, **29**, 553 (1957)
66. Downing, S. E., *Yale J. Biol. and Med.*, **30**, 43 (1957)
67. Drenckhahn, F. O., *Arch. ges. Physiol.*, **266**, 231 (1958)
68. Dubois, A. B., and Marshall, R., *J. Clin. Invest.*, **36**, 1566 (1957)
69. Earle, R. H., Perkins, J. F., Jr., and Adams, W. E., *Anesthesiology*, **19**, 153 (1958)
70. Eckenhoff, J. E., and Helrich, M., *Anesthesiology*, **19**, 240 (1958)
71. Eckenhoff, J. E., Helrich, M., and Rolph, W. D., Jr., *Anesthesiology*, **18**, 703 (1957)
72. Eckenhoff, J. E., Helrich, M., and Rolph, W. D., Jr., *Anesthesiology*, **18**, 891 (1957)
73. Edmondson, W., *Brit. J. Anaesthesia*, **29**, 570 (1957)
74. Efron, R., and Kent, D. C., *Arch. Neurol. Psychiat.*, **77**, 575 (1957)
75. Elam, J. O., Kerr, J. H., and Janney, C. D., *Anesthesiology*, **19**, 56 (1958)
76. Ellis, K., and Nadelhaft, J., *Am. J. Roentgenol., Radium Therapy Nuclear Med.*, **78**, 444 (1957)
77. Estes, E. H., Jr., Sieker, H. O., McIntosh, H. D., and Kelser, G. A., *Circulation*, **16**, 179 (1957)
78. Farhi, L. E., *J. Appl. Physiol.*, **11**, 139 (1957)
79. Ferencz, C., and Dammann, J. F., Jr., *Circulation*, **16**, 1046 (1957)
80. Ferguson, D. J., and Berkas, E. M., *Circulation Research*, **5**, 310 (1957)
81. Fishman, A. P., Turino, G. M., and Bergofsky, E. H., *Am. J. Med.*, **23**, 333 (1957)
82. Forbes, J. A., *Brit. Med. J.*, **II**, 798 (1958)
83. Forster, R. E., Roughton, F. J. W., Cander, L., Briscoe, W. A., and Kreuzer, F., *J. Appl. Physiol.*, **11**, 277 (1957)
84. Foster, C. A., Heaf, P. J. D., and Semple, S. J. G., *J. Appl. Physiol.*, **11**, 383 (1957)
85. Frahm, M., *Arch. exptl. Pathol. Pharmacol.*, **232**, 286 (1957)
86. Frank, N. E., Mead, J., and Ferris, B. G., Jr., *J. Clin. Invest.*, **36**, 1680 (1957)
87. Fritts, H. W., Jr., Harris, P., Clauss, R. H., Odell, J. E., and Cournand, A., *J. Clin. Invest.*, **37**, 99 (1958)
88. Froeb, M. F., Leftwich, C. J., and Motley, H. L., *Am. Rev. Tuberc. Pulmonary Diseases*, **77**, 737 (1958)
89. Galdston, M., and Geller, J., *Am. J. Med.*, **23**, 183 (1957)
90. Galletti, P. M., Salisbury, P. F., and Rieben, A., *Circulation Research*, **6**, 275 (1958)
91. Ghiringhelli, G., and Bosio, E., *Arch. fisiol.*, **57**, 82 (1957)

92. Goldberg, M. G., and Wolman, M., *Arch. Pathol.*, **65**, 263 (1958)
93. Graham, T. D. P., and Parker, W. A., *Quart. J. Med.*, **17**, 153 (1948)
94. Gray, J. S., *Pulmonary Ventilation and its Physiological Regulation*, 11-12, (Charles C Thomas, Publisher, Springfield, Ill., 1950)
95. Greene, R., *Brit. Med. J.*, **II**, 1028 (1957)
96. Greer, J. R., and Donald, I., *Brit. J. Anesthesia*, **30**, 32 (1958)
97. Gruhitz, C. C., *J. Pharmacol. Exptl. Therap.*, **120**, 399 (1957)
98. Haber, E., Kohn, K., Ngai, S. H., Holaday, D. A., and Wang, S. C., *Am. J. Physiol.*, **190**, 350 (1957)
99. Hackney, J. D., Sears, C. H., and Collier, C. R., *J. Appl. Physiol.*, **12**, 425 (1958)
100. Hall, F. G., and Sappenfield, L. C., Jr., *J. Aviation Med.*, **28**, 397 (1957)
101. Hall, F. G., and Zechman, F., Jr., *Proc. Soc. Exptl. Med.*, **96**, 329 (1957)
102. Halmagyi, D. F. J., Kovacs, A., and Neumann, P., *Diseases of Chest*, **33**, 285 (1958)
103. Hammond, J. D. S., *Clin. Sci.*, **16**, 481 (1957)
104. Harboe, M., *Acta Physiol. Scand.*, **40**, 248 (1957)
105. Harboe, M., and Lorentzen, F. V., *Acta Physiol. Scand.*, **41**, 77 (1957)
106. Harden, K. A., Young, R. C., Washington, W., Terry, N., and Carr, C., *J. Natl. Med. Assoc.*, **50**, 31 (1958)
107. Harkhurim, I. M., and Vigalok, S. G., *Ark. Patol. Moskva, U.S.S.R.*, **19**, 29 (1957)
108. Harrison, R. W., Adams, W. E., Beuhler, W., and Long, E. T., *Arch. Surg.*, **75**, 546 (1958)
109. Hemingway, A., Bors, E., and Hobby, R. P., *J. Clin. Invest.*, **37**, 773 (1958)
110. Hickam, J. B., and Frayser, R., *J. Clin. Invest.*, **37**, 567 (1957)
111. Holaday, D. A., Ma, D., and Papper, E. M., *J. Clin. Invest.*, **36**, 1121 (1957)
112. Honda, Y., Nomura, H., and Minoguchi, M., *Japan. J. Physiol.*, **7**, 137 (1957)
113. Hormia, A. L., *Am. J. Med. Sci.*, **233**, 635 (1957)
114. Howell, J. B. L., and Peckett, B. W., *J. Physiol. (London)*, **136**, 1 (1957)
115. Hsia, D. Y. Y., Peterson, H. G., Jr., and Gellis, S. S., *Pediatrics*, **20**, 234 (1957)
116. Hugh-Jones, P., *Brit. J. Anesthesia*, **30**, 107 (1958)
117. Hull, W. E., *Am. J. Physiol.*, **190**, 361 (1957)
118. Hume, K. M., and Gandevia, B., *Thorax*, **12**, 276 (1957)
119. Hutt, B. K., Horvath, S. M., and Spurr, G. B., *J. Appl. Physiol.*, **12**, 297 (1958)
120. Isenschmid, H., Bühlmann, A., and Schaub, F., *Helv. Med. Acta*, **24**, 82 (1957)
121. James, L. S., and Rowe, R. D., *J. Pediat.*, **51**, 5 (1957)
122. Johnson, R. P., Gold, A. J., and Freeman, G., *Am. J. Physiol.*, **192**, 581 (1958)
123. Jones, R. H., Ellicott, M. F., Cadigan, J. B., and Gaensler, E. A., *J. Lab. Clin. Med.*, **51**, 553 (1958)
124. Jordan, P., Jr., Tolstedt, G. E., and Beretta, F. F., *J. Thoracic Surg.*, **35**, 411 (1958)
125. Kamener, R., Becklake, M. R., Goldman, H., and McGregor, M., *Am. Rev. Tuberc. Pulmonary Diseases*, **77**, 209 (1958)
126. Kase, Y., and Borison, H. L., *J. Pharmacol. Exptl. Therap.*, **122**, 215 (1958)
127. Kautsky, E., *Helv. Physiol. Acta*, **15**, 358 (1957)
128. Kellogg, R. H., Pace, N., Archibald, E. R., and Vaughan, B. E., *J. Appl. Physiol.*, **11**, 65 (1957)
129. Konar, N. R., Sengupta, A. N., and Chakrabarti, B., *J. Indian Med. Assoc.*, **29**, 94 (1957)
130. Kory, R. C., *Diseases of Chest*, **33**, 465 (1958)
131. Kory, R. C., Pribek, R. A., and Sternlieb, R. O., *Am. Rev. Tuberc. Pulmonary Diseases*, **77**, 729 (1958)

132. Kovach, J. C., *Diseases of Chest*, **32**, 39 (1957)
133. Kreuzer, F., Watson, T. R., Jr., and Ball, J. M., *J. Appl. Physiol.*, **12**, 65 (1958)
134. Kuida, H., Hinshaw, L. B., Gilbert, R. P., and Visscher, M. B., *Am. J. Physiol.*, **192**, 335 (1958)
135. La Barre, J., *Thérapie*, **12**, 389 (1957)
136. Lanari-Zubiaur, F. J., and Hamilton, W. F., *Circulation Research*, **6**, 289 (1958)
137. Landmesser, C. M., Cobb, S., Peck, A. S., and Converse, J. G., *Anesthesiology*, **18**, 807 (1957)
138. LaPalme, J., *Union méd. Canada*, **86**, 973 (1957)
139. Lategola, M. T., *Am. J. Physiol.*, **192**, 613 (1958)
140. Laurent, D., Larrain, G., Singer, D., Pick, R., and Katz, L. N., *Am. J. Physiol.*, **191**, 431 (1957)
141. Lendrum, B. L., and Lichtman, A. M., *Circulation*, **16**, 1090 (1957)
142. Leusen, I., Demeester, G., and Bouckaert, J. J., *Acta Physiol. et Pharmacol. Neerl.*, **6**, 43 (1957)
143. Lewis, B. M., Forster, R. E., and Beckman, E. L., *J. Appl. Physiol.*, **12**, 57 (1958)
144. Lillington, G. A., Anderson, M. W., and Brandenburg, R. O., *Diseases of Chest*, **32**, 1 (1957)
145. Lloyd, B. B., Jukes, M. G. M., and Cunningham, D. J. C., *Quart. J. Exptl. Physiol.*, **43**, 214 (1958)
146. Loeschke, H. H., *Helv. Physiol. et Pharmacol. Acta*, **15**, c25 (1957)
147. Loofbourrow, G. N., Wood, W. B., and Baird, I. L., *Am. J. Physiol.*, **191**, 411 (1957)
148. Luchsinger, P. C., Moser, K. M., Bühlmann, A., and Rossier, P. H., *Am. Heart J.*, **54**, 106 (1957)
149. Lyons, H. A., *J. Am. Med. Assoc.*, **165**, 1939 (1957)
150. Mackay, A., Trépanier, A., and Dufresne, M. R., *J. Am. Med. Assoc.*, **166**, 1155 (1958)
151. Margaria, R., Taglietti, A., and Agostoni, E., *J. Appl. Physiol.*, **11**, 235 (1957)
152. Marshall, R., *Clin. Sci.*, **16**, 507 (1957)
153. Marshall, R., *J. Clin. Invest.*, **37**, 394 (1958)
154. Martin, C. J., and Young, A. C., *J. Appl. Physiol.*, **11**, 371 (1957)
155. Massion, W. H., *J. Appl. Physiol.*, **11**, 309 (1957)
156. Matthes, K., and Ulmer, W., *Deut. Arch. klin. Med.*, **204**, 284 (1957)
157. Matthes, K., and Ulmer, W., *Deut. Arch. klin. Med.*, **204**, 298 (1957)
158. Matzen, R. N., *Am. J. Physiol.*, **190**, 84 (1957)
159. May, G., Phillips, M., and Adriani, J., *Anesthesiology*, **18**, 871 (1957)
160. May, P., *Helv. Physiol. et Pharmacol. Acta*, **15**, 230 (1957)
161. Megirian, R., and White, C. W., *New Engl. J. Med.*, **257**, 849 (1957)
162. Mendel, D., and McIlroy, M. B., *Brit. Heart J.*, **19**, 399 (1957)
163. Mercier, J., and Shaw, F. H., *Thérapie*, **12**, 493 (1957)
164. Metz, B., *Am. J. Physiol.*, **192**, 101 (1958)
165. Michelson, A. L., and Lowell, F. C., *Am. J. Med. Sci.*, **234**, 31 (1957)
166. Michelson, A. L., and Lowell, F. C., *Am. J. Med.*, **24**, 225 (1958)
167. Mills, H., and Kattus, A. A., Jr., *Circulation*, **17**, 65 (1958)
168. Miller, C. A., and Reed, H. R., *Pediatrics*, **21**, 362 (1958)
169. Miller, H. C., *Pediatrics*, **20**, 817 (1957)
170. Motley, H. L., *Am. Rev. Tuberc. Pulmonary Diseases*, **76**, 601 (1957)
171. Murphy, J. P., *Circulation*, **17**, 199 (1958)
172. Ngai, S. H., *Am. J. Physiol.*, **190**, 356 (1957)
173. Ngai, S. H., and Wang, S. C., *Am. J. Physiol.*, **190**, 343 (1957)

174. Nims, R. G., Conner, E. H., and Comroe, J. H., Jr., *J. Clin. Invest.*, **34**, 744 (1955)
175. Oberholzer, R. J. H., and Schlegel, H., *Helv. Physiol. et Pharmacol. Acta*, **15**, 63 (1957)
176. Paff, G. H., and Boucek, R. J., *Circulation Research*, **6**, 88 (1958)
177. Palva, T., Elo, R., and Saloheimo, M., *Diseases of Chest*, **32**, 56 (1957)
178. Pauli, H. G., Noe, F. E., and Coates, E. O., Jr., *Henry Ford Hosp. Med. Bull.*, **5**, 119 (1957)
179. Perkins, J. F., Jr., Adams, W. E., Flores, A., Harper, P. V., and Landahl, H. D., *J. Appl. Physiol.*, **12**, 71 (1958)
180. Pesiri, E. J., and Sennott, W. M., *Diseases of Chest*, **31**, 548 (1957)
181. Peters, R. M., *Am. J. Physiol.*, **191**, 399 (1957)
182. Pitts, R. F., Magoun, H. W., and Ranson, S. W., *Am. J. Physiol.*, **126**, 673 (1939)
183. Pryor, W. W., Hickam, J. B., Sieker, H. O., and Page, E. B., *Circulation*, **15**, 721 (1957)
184. Quimby, C. W., Jr., Aviado, D. M., Jr., and Schmidt, C. F., *J. Pharmacol. Exptl. Therap.*, **122**, 396 (1958)
185. Rayl, J. E., and Smith, D. E., *Diseases of Chest*, **33**, 235 (1958)
186. Redgate, E. S., and Gellhorn, E., *Am. J. Physiol.*, **193**, 189 (1958)
187. Reichert, J. L., *Postgrad. Med.*, **22**, 142 (1957)
188. Renzetti, A. D., Jr., and Padget, W. R., *J. Lab. Clin. Med.*, **50**, 400 (1957)
189. Rimini, R., Duomarco, J. L., Burgos, R., Dighiero, J. C., Saprizza, J. P., and Surraco, G. H., *Diseases of Chest*, **31**, 643 (1957)
190. Rivera-Estrada, C., Saltzman, P. W., Singer, D., and Katz, L. N., *Circulation Research*, **6**, 10 (1958)
191. Rochford, J., Welch, R. F., and Winks, D. P., *Brit. J. Anaesthesia*, **30**, 23 (1958)
192. Rose, J. C., and Lazaro, E. J., *Circulation Research*, **6**, 283 (1958)
193. Roughton, F. J. W., and Forster, R. E., *J. Appl. Physiol.*, **11**, 290 (1957)
194. Rowe, R. D., and James, L. S., *J. Pediatr.*, **51**, 1 (1957)
195. Rudolph, A. M., and Paul, M. H., *Am. J. Physiol.*, **189**, 263 (1957)
196. Salisbury, P. F., Weil, P., and State, D., *Circulation Research*, **5**, 303 (1957)
197. Samet, P., Rosenthal, A., and Bernstein, W. H., *Am. J. Med.*, **24**, 215 (1957)
198. Schaumann, W., *Arch. Exptl. Pathol. Pharmacol.*, **233**, 98 (1958)
199. Scherrer, V. M., and Bucher, U., *Helv. Med. Acta*, **24**, 385 (1957)
200. Schmitt, G. H., and Meyers, F. H., *Am. J. Physiol.*, **190**, 89 (1957)
201. Sergievski, M. V., and Ivanov, I. N., *Fiziol. Zhur. S.S.S.R.*, **44**, 126 (1958)
202. Severinghaus, J. W., and Stupfel, M., *J. Appl. Physiol.*, **10**, 335 (1957)
203. Severinghaus, J. W., Stupfel, M., and Bradley, A. F., *J. Appl. Physiol.*, **10**, 349 (1957)
204. Sharp, J. T., Griffith, G. T., Bunnell, I. L., and Greene, D. G., *J. Clin. Invest.*, **37**, 111 (1958)
205. Shepard, R. H., *J. Appl. Physiol.*, **12**, 487 (1958)
206. Shepard, R. H., Campbell, E. J. M., Martin, H. B., and Enns, T., *J. Appl. Physiol.*, **11**, 241 (1957)
207. Shephard, R. J., Carey, G. C. R., and Phair, J. J., *J. Appl. Physiol.*, **12**, 79 (1958)
208. Simmons, D. H., and Hemingway, A., *Am. Rev. Tuberc. Pulmonary Diseases*, **76**, 195 (1957)
209. Singer, D., Hesser, C., Pick, R., and Katz, L. N., *Circulation Research*, **6**, 4 (1958)
210. Singer, D., Saltzman, P. W., Rivera-Estrada, C., Pick, R., and Katz, L. N., *Am. J. Physiol.*, **191**, 437 (1957)

211. Spurr, G. B., Hutt, B. K., and Horvath, S. M., *J. Appl. Physiol.*, **11**, 58 (1957)
212. Stahlman, M. T., *J. Clin. Invest.*, **36**, 1081 (1957)
213. Stamm, W., and Bucher, K., *Acta Physiol. et Pharmacol. Neerl.*, **6**, 261 (1957)
214. Steinberg, S. S., Bellville, J. W., and Seed, J. C., *J. Pharmacol. Exptl. Therap.*, **121**, 71 (1957)
215. Storstein, O., Helle, J., and Rokseth, R., *Am. Heart J.*, **55**, 781 (1958)
216. Svanberg, L., *Scand. J. Clin. & Lab. Invest.*, **9**, Suppl. 25, 1 (1957)
217. Swan, H. J. C., Marshall, H. W., and Wood, E. H., *J. Clin. Invest.*, **37**, 202 (1958)
218. Swerdlow, M., *Brit. J. Anaesthesia*, **30**, 2 (1958)
219. Takezawa, H., *Japan. Circulation J.*, **21**, 335 (1957)
220. Takezawa, H., *Japan. Circulation J.*, **21**, 583 (1958)
221. Takezawa, H., *Japan. Circulation J.*, **21**, 588 (1958)
222. Takezawa, H., *Japan. Circulation J.*, **21**, 621 (1958)
223. Takizawa, T., and Haseyama, H., *Tohoku J. Exptl. Med.*, **66**, 24 (1957)
224. Tang, P. C., Maire, F. W., and Amassian, V. E., *Am. J. Physiol.*, **191**, 218 (1957)
225. Taquini, A. C., Roncoroni, A. J., Aramendia, P., and Ros, A. M., *Am. Heart J.*, **54**, 319 (1957)
226. Taylor, H. J., *Nature*, **180**, 883 (1957)
227. Theron, J. C., Zwi, S., and McGregor, M., *Lancet*, **II**, 415 (1958)
228. Van Fossan, D. D., and Clark, R. T., Jr., *Am. J. Physiol.*, **192**, 577 (1958)
229. Vidone, R. A., and Liebow, A. A., *Am. J. Physiol.*, **33**, 539 (1957)
230. Viikari, S. J., and Autio, V., *Acta Med. Scand.*, **157**, 61 (1957)
231. Walley, R. V., *Lancet*, **I**, 1143 (1957)
232. Wang, S. C., Ngai, S. H., and Frumin, M. J., *Am. J. Physiol.*, **190**, 333 (1957)
233. Weakley, L. S., and Bergner, R. P., *Anesthesiology*, **18**, 603 (1957)
234. Wégria, R., *Am. J. Med.*, **19**, 509 (1955)
235. Weil, P., Salisbury, P. F., and State, D., *Am. J. Physiol.*, **191**, 453 (1957)
236. West, J. B., Fowler, K. T., Hugh-Jones, P., and O'Donnell, T. V., *Clin. Sci.*, **16**, 529 (1957)
237. West, J. B., Fowler, K. T., Hugh-Jones, P., and O'Donnell, T. V., *Clin. Sci.*, **16**, 548 (1957)
238. Westling, H., *Acta Physiol. Scand.*, **40**, 75 (1957)
239. Whitteridge, D., and Bulbring, E., *J. Pharmacol. Exptl. Therap.*, **81**, 340 (1944)
240. Williams, M. H., Jr., *N. Y. State J. Med.*, **57**, 2673 (1957)
241. Williams, M. H., Jr., *Diseases of Chest*, **32**, 656 (1957)
242. Williams, T. F., Winters, R. W., Clapp, J. R., Hollander, W., Jr., and Welt, L. G., *Am. J. Physiol.*, **193**, 181 (1958)
243. Wilson, R. H., Jay, B. E., Meador, R. S., and Evans, R., *Am. J. Med. Sci.*, **234**, 547 (1957)
244. Winters, R. W., Lowder, J. A., and Ordway, N. K., *J. Clin. Invest.*, **37**, 640 (1958)
245. Woolmer, R., *Brit. Med. Bull.*, **14**, 54 (1958)
246. Wyss, O. A. M., *Acta Physiol. et Pharmacol. Neerl.*, **6**, 685 (1957)
247. Yamamoto, W. S., *Am. J. Physiol.*, **191**, 423 (1957)
248. Youmans, W. B., and Schopp, R. T., *Proc. Soc. Exptl. Biol. Med.*, **95**, 100 (1957)
249. Young, I. M., *J. Physiol. (London)*, **137**, 374 (1957)
250. Yu, P. N., Nye, R. E., Jr., Lovejoy, F. W., Jr., Schreiner, B. F., and Yim, B. J. B., *J. Clin. Invest.*, **37**, 194 (1958)
251. Zechman, F. W., Jr., Salzano, J., and Hall, F. G., *J. Appl. Physiol.*, **12**, 301 (1958)
252. Zuniga-Caro, A., and Orrego-Puelma, H., *Diseases of Chest*, **32**, 534 (1957)

DIGESTIVE SYSTEM¹

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To understand digestion we need first a gross, qualitative description of the fate of ingesta: what is secreted in response to the meal, how ingesta are transformed by reaction with secretions and by the propulsive mechanism, what is absorbed and what rejected. This should be followed by an exact quantitative and temporal description in the style of chemical kinetics in which net changes are reported in terms of two-way fluxes. Coupled with this should be a detailed account of the physiological and morphological basis of secretion, transformation, and absorption reaching down to the deepest levels at which anatomy, biochemistry, and biophysics become indistinguishable one from another. Finally we require a description complete at all levels of controlling processes. Then we can put the fragments of our knowledge together to characterize what happens in the gut.²

Descriptive, analytical, and synthetic aspects of our knowledge of digestion are incomplete, for gastroenterological physiology suffers from difficulties inherent in all biological science. Even summary descriptions must frequently be revised as we gain new tools and insights, and scientific attack does not proceed in an orderly manner. Before we had learned to describe digestion in simple terms there were triumphs of analysis, as for example the discovery of the secretin mechanism, but we cannot say how individual parts cooperate to perform the total function. In addition, gastroenterological physiology has its peculiar troubles. What is ingested may range from a ten course dinner served at the Duchess of Guermantes' to repulsive mixtures of mud and mush appropriately called "test meals." There are wide species differences, and even within one species the digestive apparatus shows great functional variability. Consequently, when we come to describe what actually occurs during digestion we must specify what meal was eaten by what animal under what circumstances.

SECRETION FROM THE SALIVARY GLANDS

Not only do salivary glands differ from species to species, but several glands in one animal secrete juices of different compositions. The parotid

¹ This review considers selected aspects of the recent literature available in the University of Michigan library at the end of May, 1958.

² Papers on the following phases of digestion are cited for completeness but will not be discussed: on secretion (161 to 172); on the pancreas (173 to 177); on bile (178 to 182); on absorption (183 to 199); on cholesterol absorption (200 to 210); on neurophysiology (211 to 217); on smooth muscle (218 to 220); on the esophagus (221 to 228); on regulation (218 to 229); on the effects of drugs and 5-hydroxytryptamine (229 to 242); on growth, injury, and repair (243 to 249); on blood flow (250 to 255); and on methods (257 to 263).

gland of dog and man secretes hypotonic juice the composition of which varies with rate of secretion. Burgen (1) at the beginning of his systematic study of salivary secretion resolved parotid water into isotonic water and free water and examined two hypotheses: (a) that the gland secretes water at one locus and an isotonic solution at another with the latter dominating at high rates of flow, and (b) that isotonic secretion occurs in the acini with solute reabsorption in the ducts. Since the ducts are probably permeable to water, the high osmotic gradient between saliva and extracellular fluid would cause water reabsorption to occur there. The ducts have a large blood flow, of the order of 20 to 30 ml. per gm. duct per min. (2), almost equal to total gland blood flow. Burgen's (1) studies of parotid secretion of non-electrolytes showed that at low rates of secretion some (e.g. urea, thiourea, methylurea) are more concentrated in juice than in blood or glandular tissue, and from these data he calculates an "osmotic reabsorption coefficient" of about 21 mg. H_2O per gm. per Osmol. per min. If the reviewer's arithmetic is correct and if the Van't Hoff equation holds, this is to be compared with a value of 2800 in the same units for mammalian muscle capillaries (3). Iodide and thiocyanate are concentrated in cells of the duct segment of the parotid (4), and if concentration within cells can be taken as a sign of secretion (a dangerous assumption) we must regard parotid ducts as having functions as complex as the renal tubules, both secreting into and reabsorbing from the fluid presented to them by the acini.

Lundberg (5) has reviewed his own microelectrode work which is beginning to explain both electrical signs of salivary secretion and some functions of acinar cells. Three types of potentials may be recorded from electrodes inserted in cat submaxillary glands. (a) There is a resting potential, extracellular positive, of about 22 mv. On either parasympathetic or sympathetic stimulation, hyperpolarization of about 30 mv. occurs. The time course depends upon the mode and frequency of stimulation. (b) Other cells on the surface of the gland having resting potentials of 35 mv. show hyperpolarization after parasympathetic and depolarization after sympathetic stimulation. (c) Deep in the gland are cells with resting potentials of 80 mv. whose potential decays slowly to 10 to 20 mv. after either mode of stimulation. Lundberg, on the basis of the locus from which these three types can be recorded, attributes them to the alveolar, semilunar, and duct cells. Potentials across the apical membranes in this gland cannot be deduced and have not been measured. In the histologically simpler cat sublingual gland, recordings from intracellular electrodes and from the lumen of the duct show a resting potential of the basal membrane of 33 mv. extracellular positive, and an apical potential of same sign a few mv. higher. Two seconds after beginning of repetitive parasympathetic stimulation the basal potential rises to 56 mv., and later the apical potential may rise to 43 mv. These changes, called secretory potentials, are independent of the absolute value of the resting potential which may be artificially set at almost any value. The two membranes are electrically inexcitable, and secretory potentials produced either

by nervous stimulation or by perfusion with the transmitter can be graded. The membranes are ohmic resistances, and that of the basal, but not of the apical, membrane falls upon stimulation. When the sublingual gland is perfused with oxygenated salt solution containing chloride, normal secretory potentials are obtained. Reduction of chloride content below 50 per cent, replacing it with nitrate, is followed by a reduction of secretory potentials, and when chloride is completely replaced the secretory potential is 10 to 25 per cent of maximum. Bromide can substitute for chloride, but iodide and thiocyanate behave like nitrate. Lundberg's conclusions are sweeping, for he has been to Canberra. A chloride pump residing in the basal membrane is activated by the transmitter, and its forced movement of chloride from extracellular fluid through the cell and apical membrane is largely the cause of secretion of salt and water. How justified is doubt of the ultimate correctness of this hypothesis is shown by Burgen's (1) work on secretion of nonelectrolytes. Using ideas and techniques derived from Höber but elaborated with great sophistication, he has measured secretion of ten nonelectrolytes in dog parotid saliva. These vary fivefold in molecular weight and 100,000-fold in oil/water solubility coefficients. There are two classes of relations between their concentration in saliva and volume rate of secretion: (a) those (e.g., methenamine) which fall off logarithmically as rate rises, and (b) those (e.g., N-methylurea) which reach a minimum and rise again. There are wide differences in concentration ratios ranging from 70 to 100 per cent of plasma concentration (N-ethylurea) to 2 to 4 per cent (mannitol). Burgen calculates permeation constants on the assumption that the substances move across the cells by diffusion into a solution free of them. The constant should be independent of volume rate of flow, and for some (ethylurea, chloramphenicol, etc.) it is. For others (urea, creatinine, etc.) it rises steeply with volume rate of flow, and Burgen concludes that in the resting gland permeation is through lipid material in the cell wall but that with activity there is a large change in cell permeability as the result of opening of water-filled pores. Because concentrations of his test substances are approximately equal in gland and plasma, Burgen believes this change in permeability occurs at the basal membrane. This would account for the lowering of resistance observed by Lundberg; but if a general increase in permeability occurs, its electrical sign, or the secretory potential, might have almost any value depending upon the relative concentrations and ionic conductances.

When salivary secretion begins there is a transient loss of potassium from cells into both secretion and venous blood (1a). Burgen (2) has given an explanation for the transient together with a comprehensive explanation of salivary secretion. If one measures the specific activity of radioactive substances (^{42}K , ^{14}C -urea, ^{22}Na) in the gland, its secretion, and in venous blood draining the gland one can distinguish between two processes: (a) if saliva collected is the primary secretion of the cells, the specific activity of the salivary constituents should equal that in the secreting cells, but (b) if secondary equilibration occurs within the ducts, the specific activity of the

saliva should be lower. For ^{42}K the specific activity of the first few drops of saliva is nearly equal to that in the cells, but thereafter it falls off. For both urea and sodium, salivary specific activity is also lower. Burgen thinks the primary secretion from acinar cells is an isotonic solution high in potassium and bicarbonate, low in sodium, and intermediate in chloride. Although transport of such a solution through the cells would generate potentials such as those observed by Lundberg, Burgen believes the chief work of the cells is accumulation of potassium, not transport of chloride. When this solution is in the ducts, potassium diffuses rapidly from it into the blood but sodium diffuses into it much less readily. Differential diffusion coupled with low permeability to water accounts for the hypotonicity of the juice. Because blood supply to the ducts is in series with that to the acini and flow is countercurrent (there being some anatomical evidence for this deduction), potassium is picked up by the acini and recycled. Urea is low in the primary secretion, and about two-thirds of juice urea is added in the ducts. Two other factors must be considered: (a) the effect of rate of flow in the ducts upon time for equilibration, and (b) increase in permeability of the ducts, for unknown reasons, with increasing rate of flow. With increased flow, sodium and chloride concentrations rise, so the second factor must be overwhelmingly dominant. Burgen calculates that ductal permeability to urea increases about tenfold; and since this implies opening of water-filled channels, permeability to electrolytes should also rise.

In parotid or submaxillary secretion of the dog, the saliva to plasma ratio of lithium is two to three at low rates but approaches one at higher rates. There is no secretory transient; the ratio is independent of plasma level; the gland does not accumulate lithium. Burgen disappoints by not suggesting an explanation (1b).

The sheep parotid gland secretes 1 to 4 l. per day of alkaline (pH 8.2), hypertonic (ca. 385mOsm.) saliva containing sodium above plasma level (ca. 167 mM), high potassium (ca. 25 mM), high bicarbonate, and variable chloride. In normal animals the Na:K ratio may be as high as 180:10. Denton (6) and colleagues have described a method of preparing a permanent parotid fistula, and using this preparation they have studied fully the effects of loss of parotid juice on secretion itself and on the animal. There is a basal flow of saliva. Upon stimulation there are transients similar to those found by Burgen (1a) in the dog, and during established activity there is a regular relation between ionic composition and rate, increased flow being associated with increase in sodium and bicarbonate and decrease in potassium and phosphate. Osmolarity as determined by adding up the ions does not change. If the juice is lost, the sheep rapidly experiences a depletion of 400 to 600 m.eq. of sodium with the expected results on its fluid compartments (6a, 8). The volume of flow decreases, sodium falls to 60 m.eq., and potassium rises to 120 m.eq. The Na:K ratio may go to 10:180. Denton and colleagues (6, 9, 10) have shown that although there is a small local effect of changed blood flow, reduced secretion rate, and plasma ionic composition

upon the parotid gland the major part of the reversal of Na:K ratio is mediated through the adrenal glands. In absence of adrenals the Na:K ratio does not change as the animal becomes depleted and secretion rate falls. When the glands are intact, depletion alters their hormone output so that the parotid glands can reduce their sodium secretion and raise that of potassium. There is no hint of the cellular mechanism of this spectacular effect, but Burgen (2) suggests that saliva of depleted sheep may actually represent the primary secretion from acinar cells and that adrenal deficiency, by altering permeability of the ducts, prevents secondary changes. Another unexplained effect of hormones is that although the normal salivary glucose threshold in dogs is about 27 mM it is reduced to about 15 mM in alloxan diabetes and raised to about 67 mM by administration of insulin (11).

Acetazoleamide causes a statistically significant but physiologically dubious rise in human salivary calcium concentration, and studies on composition of human saliva (12 to 15) demonstrate chiefly that meaningful data are hard to obtain on account of variations from gland to gland and person to person.

GASTRIC SECRETION

Among the numerous proteins of gastric juice are pepsinogen, cathepsin, and lipase. The lipase is tributyrase, having almost no lytic activity on fats containing longer chain lengths. Plasma lipase is also tributyrase (16) and the reviewer hesitates to suggest that all the effort spent in study of plasma pepsinogen be duplicated in a study of plasma tributyrase. Until recently cathepsin was merely a hump on the pH-activity curve (17), and considering problems raised by buffer concentration, ionization of substrate, and so forth most critical biochemists have regarded it as an artifact. Now Richmond *et al.* (18) have completely separated small amounts of cathepsin from human gastric juice, and Miller *et al.* (19) have adduced further evidence that two proteolytic enzymes are secreted by the mucosa and excreted in urine at different rates. The physiological significance of the second enzyme having a pH-activity peak at 3.5 has not been determined. Hirschowitz's (20) review of pepsinogen summarizes most of the current knowledge, sometimes from an individual point of view, and it contains information on comparative physiology. Histamine does stimulate pepsinogen secretion in man, however doubtful its action may be in the dog, for histamine can induce high rates of secretion for many hours (21). In general, pepsinogen, acid, and chloride secretion go together in man, but they can be dissociated as for example by inhibitors of acid secretion such as acetazoleamide. As the gastric mucosa atrophies, acid secretion fails before pepsinogen secretion which in turn is survived by secretion of intrinsic factor (22, 23). Small amounts of pepsinogen appear in plasma and urine, and there have been many attempts to attach diagnostic significance to the levels. Plasma pepsinogen does come from the gastric mucosa, for its concentration increases in gastric venous blood of dogs when secretion is stimulated (24). It has been assumed that during gas-

tric secretion a certain fraction of secreted pepsinogen for unknown reasons finds its way into plasma and then is quantitatively excreted in urine so that uropepsin is a measure of gastric secretion. There have been innumerable measurements of plasma and urine pepsinogen under many circumstances ranging from a 15-hr. night flight (25) to the third trimester of pregnancy (26). There is a rough correlation between the ability of the mucosa to secrete pepsinogen and the plasma level, for at high rates of gastric secretion plasma pepsinogen is up, and when massive stimulation of secretion occurs, as for example in guinea pigs given a histamine dose of 75 mg./kg. (!) plasma, pepsinogen may increase sixfold (27). At lower levels there is poor correlation. If plasma pepsinogen does not reflect secretion, perhaps, as Hirschowitz (20) believes, it comes from disintegrating chief cells. The relation between gastric secretion and urinary pepsinogen is even rougher. Proteins of relatively low molecular weight are filtered in the glomeruli, and large but variable fractions present in tubular urine are reabsorbed. Consequently, rate of excretion depends not only on rate at which the protein is delivered to the kidney but also on filtration and reabsorption. In five male medical students, Hirschowitz & Streeten (28) found that infusion of 25 clinical units of ACTH every 12 hrs. for five to six days caused a rise in urinary pepsinogen clearance without substantial changes in gastric pepsinogen output or plasma concentration. If the unreliable endogenous creatinine clearance can be taken as a measure of glomerular filtration rate, increased excretion was the result of decreased tubular reabsorption. Finally, two careful clinical studies (29, 30) have shown that although, on the average, plasma and urine pepsinogen levels are higher in patients with duodenal ulcers (and hence presumably with higher secretory rates), single determinations of either value are of no differential diagnostic significance on account of great variations from person to person and from day to day. There may be a tenfold difference in daily uropepsin output between individuals in the same clinical status (31).

There is little new on gastric acid secretion, all that is known on metabolic aspects being covered in the 1955 University of Wisconsin symposium (32). The flux of chloride from nutrient to secretory sides of frog gastric mucosa is not surprisingly increased when the mucosa is stimulated to secrete (33). When metabolic inhibitors depress acid secretion chloride moves through the mucosa as a free ion, but during secretion measured chloride conductance is less than calculated from the mass actually moving, chloride then being bound to an imaginary carrier whose supply depends on metabolism. In addition to that accompanying acid, chloride also moves from nutrient to secretory side as result of an emf.-generating process which could account for the electrical energy produced by the mucosa (34). If the electrical energy production can be used as measure of the second kind of chloride pumping, it can be separated from acid secretion. It is unchanged in the face of variations in acid secretion over a wide range. Thiocyanate suppresses acid secretion without affecting the chloride pump, and a combination of acetazoleamide and histamine may abolish the gastric emf. without affecting secretion.

Dissociation of emf. and acid secretion, if substantiated, has great theoretical importance; but to accept the conclusion, based on the use of acetazoleamide in unstated concentration on a tissue in which metabolic rate, carbon dioxide production, and the uncatalyzed rate of hydration are all unknown, that carbonic anhydrase is necessary for the chloride pump but not for acid secretion requires simple faith in specificity of inhibitors and ignorance of the unhappy history of that enzyme. In man at least, very high doses of acetazoleamide reversibly inhibit acid secretion 80 to 100 per cent for 2 to 8 hr. (35). The gastric potential difference is not caused by the bicarbonate gradient for it is unaffected by bicarbonate on the mucosal side (36). A new explanation of the as yet inadequately characterized osmotic activity of gastric juice is elaborated on Rehm's familiar assumptions that hydrogen ions are secreted by the surface epithelial cells and chloride and water by the parietal cells, without adding any new or decisive data (37). There is a small increase in radio-phosphate specific activity of Schmidt-Tannhauser fractions of gastric mucosa of rats given 1 mg. histamine as compared with those given 1 mg. atropine, but there is no change in the RNA phosphorus (38). The further claim that RNA participates in acid secretion because saliva, on account of its ribonuclease content, inhibits frog mucosae cannot be accepted, for neither the reviewer's saliva (he does not smoke and hence has no salivary thiocyanate which would inhibit secretion) nor an authentic sample of ribonuclease inhibits his preparations. The important problem of adaptive enzyme changes in mammals, a problem still inadequately defined for kidney and pancreas, is raised for the stomach by the assertion that sympathectomy but not vagotomy increases the carbonic anhydrase content (as measured by a crude method) threefold and raises both the P:O ratio and ATP content in dog stomachs (39). Controls of variations from place to place in the mucosa were not reported.

Glass (40) continues his laborious analysis of human gastric mucoproteins, finding great differences among fractions separated by electrophoresis. Two samples of serum mucoprotein differed from all gastric samples in protein to carbohydrate ratio and carbohydrate composition, showing that serum mucoproteins are probably not derived from the stomach. Purification of intrinsic factor has reached a sample effective in 3 mg. doses and showing only three sedimentation peaks (41). Workers in Wolf's laboratory (42) in their purification of inhibitory substance found in normal human gastric juice and saliva obtained a fraction, either a small mucoprotein or a carbohydrate, which is released from the antrum and which requires vagal innervation for its secretion. It is active in small doses and has profoundly important long-term properties, review of which must unfortunately wait transfer of information from progress report and gossip to definitive publication. Attempts to measure mucoprotein synthesis using ^{35}S -sulfate and *in vitro* preparations of guinea pig gut found negligible gastric but considerable colonic synthesis (43, 44). ^{35}S -sulfate is incorporated as ester sulfate, and the process is energy-requiring.

PANCREATIC SECRETION

Problems of salivary and pancreatic protein synthesis and secretion are similar and the proteins of neither have been completely characterized. Human parotid saliva on electrophoresis shows seven (45) to twelve (46) peaks. Two account for 75 per cent of the protein, and amylase activity is distributed in two as well. When rat pancreas is fractionated by differential centrifugation, amylase follows the zymogen granules, but large amounts are in other fractions too (47). Simple centrifugation procedures are not sufficiently reliable to distinguish among fractions, for when they are controlled with electronmicroscopy the zymogen granule fraction at best is not completely pure (48). However, the trypsin-activatable proteolytic enzymes do appear to be confined to the granules. Ribonuclease is largely but perhaps not exclusively in the granules. A protective mechanism is represented by distribution of trypsin inhibitor in what centrifugers call the "cell sap". Catalase is absent from human salivary secretion and if present in the juice is of bacterial origin, but peroxidase is secreted (49). Elastase occurs in pancreatic juice of rat and guinea pig and its concentration in the gland increases or decreases in parallel with lipase (50). Protaminase which liberates only free arginine from C-terminal ends of protamine is in pig pancreatic extracts not in association with any other proteolytic enzyme (51). In the purest preparations from pig pancreatin only one or a closely related family of lipolytic proteins giving a color reaction of lipoproteins is obtained (52). Changes in concentration of enzymes in either gland probably represent *de novo* synthesis rather than activation, and the rat pancreas can secrete 23 to 27 per cent of its protein per day (53). A tenfold rise in rat salivary amylase occurs within 150 min. of stimulation by acetylcholine, and a similar rise follows feeding, both being preventable by atropine (54). *In vitro* secretion by mouse pancreas is stimulated by pilocarpine, carbachol, and pancreozymin with an accompanying increase in respiration whereas secretin stimulates only respiration (55). Neither secretory nor respiratory rate is reduced by atropine when pancreozymin is the stimulus. When rat pancreas is incubated with radioactive amino acids, the curve relating the specific activity of ribonucleic acid and time intersects the axes at zero which means either that the time of synthesis of one protein molecule is of the order of a few seconds or that amino acids may exchange with protein after synthesis is complete (56).

Total amount and concentration of enzymes in the pancreas may readily be altered by manipulation of diet (57, 58), but the vexed question of parallel secretion (Babkin) versus purposive adaptation (Pavlov-Walther) has been partially resolved in favor of neither. Guth and his co-workers (16) are to be congratulated on their papers on pancreatic secretion in the dog, characterized by careful attention to precision of method, an adequate number of observations, and penetrating statistical analysis. In the latest they show that variations in lipase secretion are not related to those in total protein nitrogen, hence no parallel secretion, and that the variations do not conform to type of diet, hence no purposive adaptation over the short run. We still need experiments of the same caliber in which unbalanced diets are fed for weeks or months.

ENTERIC ABSORPTION

The pioneer work of Visscher and his co-workers (59 to 61) on absorption of salts and water as resultant of two-way fluxes across the enteric mucosa has not been superseded, but we are in the midst of a flood of papers released by Fisher & Parsons' (62) popularization of *in vitro* methods. Absorption of anything measurable is being studied with a variety of methods and we have not yet achieved a coherent body of data which can readily be interpreted in an annual survey. The presence of glucose is required for water absorption by isolated jejunal loops, the greatest absorption occurring with glucose at the mucosal surface and itself being absorbed. However, when one-tenth isosmotic glucose is confined to the serosal surface, water absorption is at about 65 per cent of the maximal rate (63). In a preparation having no fluid initially in contact with the serosal surface, absorption of water likewise depends on glucose, oxygen, osmotic pressure, and temperature and is prevented by the usual metabolic inhibitors. The absorbate collecting on the serosal surface can contain alanine, glucose, and the lower fatty acids in concentrations higher than those in the mucosal fluid, sodium and chloride in approximately equal concentrations, and creatinine and urea in lower ones (64). As for glucose absorption itself, Riklis, Haber, and Quastel (65, 66) found that raising potassium to the optimal concentration of 15.6 m.eq. doubles the rate of glucose absorption by isolated guinea pig intestine from 14 mM glucose. The same potassium concentration also accelerates that fraction of fructose absorption depending on conversion to glucose, transport of fructose itself being unaffected. Rubidium but not caesium also stimulates, and presence of sodium and magnesium is essential. Stimulation of glucose absorption is blocked by 2 mM malonate, 0.5 mM choline, or 0.01 mM phlorrhizin. Glucose at 14 mM inhibits galactose absorption by 80 per cent. Rates of absorption of glucose and fructose, both at 14 mM, are additive but not when their concentrations are higher. Extracts of rat intestinal mucosa can phosphorylate and dephosphorylate fructose, and when fructose is being absorbed the concentration of fructose esters increases up to 14 times in the mucosa but not in the portal blood (67). A quantitatively important species difference exists in fructose absorption; for when evenly labeled ^{14}C -fructose was absorbed by jejunal loops, 10 per cent was recovered as glucose in the rat and 70 per cent in the guinea pig. In both some fructose went to lactate while unidentified ^{14}C compounds occurred to the extent of not over 3 per cent in the guinea pig but in the rat to as much as 30 per cent (68).

Rat intestine transports short-chain fatty acids from mucosal to serosal side against a small concentration gradient, and movement is abolished by anaerobiosis or DNP and reduced by absence of glucose (64). It is not clear whether this is transport of the acids themselves or movement secondary to sodium transport. The technique of Borgström and co-workers (69), perhaps combined with thoracic duct cannulation (70), promises to give a complete description of fat digestion and absorption in man. Fats are fed with two kinds of labels, ^{13}C and ^{14}C fatty acids and methyl groups on the 2 position of the acids in glycerides to prevent hydrolysis. When frequent samples are taken via transintestinal tubes, hydrolysis and absorption can be followed.

Using two assumptions Borgström calculated that about 60 per cent of fed triglyceride bonds are split before absorption, that about 40 per cent of triglycerides are completely split, and that the remaining triglycerides are partly split giving a mixture of tri-, di-, and monoglycerides and free fatty acids. The two assumptions are (a) that intestinal contents are not significantly diluted with endogenous glycerides and (b) that the different glycerides are absorbed at the same rate. There is experimental support for the first, but the second assumes a fact we want to know. Fortunately, the need for the second assumption can be eliminated by use of more labels, and in rats Tidwell (71) has shown that rates of disappearance of oleic acid and olive oil are identical if calculated on basis of actual amounts presented to the small intestine by the stomach.

When glutamic acid is being absorbed, alanine increases in mesenteric venous blood (72), showing that transamination has occurred, but it is not possible from the data to deduce what fraction of absorbed acid was metabolically attacked. Because the pyruvate concentration did not fall, that acid used must have come from cellular metabolism. Pyridoxal may be required in transamination, but added pyridoxal which increases amino acid accumulation by ascites tumor cells has no effect on transport of proline, glycine, histidine, or methionine by intestine of the golden hamster (73). Further evidence of metabolic intervention is that methionine reduces the rate of absorption of histidine (74). That it also reduces the amount of histidine present in the intestinal wall suggests that the effect is on uptake of the acid by the cell and that release into blood is a passive process.

INTESTINAL MOTILITY

Physostigmine increased the rate of glucose absorption about 15 per cent presumably by increasing peristalsis in an isolated intestine preparation and hence the amount of fluid circulated in a given time (75). Absorption of oleic acid or triolein in unanesthetized dogs varied directly with initial lipid concentration and inversely with volume of mixture traversing ileal loops (76). Atropine caused decrease in volume of contents and, therefore, increased the rate of absorption at a given initial lipid concentration. In anesthetized dogs with vagi intact, atropine first increased rate of absorption of 171 mM NaCl and then decreased it, but in vagotomized dogs atropine only increased absorption (77). Although fluxes were not measured, these changes were attributed to the effect of atropine in decreasing secretion with a consequent rise in net absorption. Signs of more complex relations between motility and absorption were found in unanesthetized rats in which motility was grossly measured by a pressure-recording catheter in the lumen of the upper small intestine and absorption by an indwelling cannula in the abdominal lymph duct (78). In fasting animals atropine reduced motility with no change in lymph flow, whereas in animals fed olive oil large changes in flow were not associated with changes in motility. These and the previously cited experiments would be more intelligible if we knew not only fluxes and blood flow but motility of the muscularis mucosae and of the villi as well.

The properties of gastrointestinal smooth muscle, the basic unit of the propulsive mechanism, are just becoming known. In vertebrate smooth muscle cells, myofilaments vary greatly in size and arrangement and they may not be grouped together in fibrils (79). They are not regularly packed, and lack of regular transverse alignment results in absence of cross striations. With no Z or M lines, filaments may slide to an unlimited extent with respect to each other so that tension may develop over a great range of lengths. The cells show lateral indentations which vary with state of contraction whereas polar indentations remain uniform. "This would mean that in contraction the cells merely fold upon themselves like an accordion (80)." It is assumed that the contractile processes of striated and smooth muscles are the same, and whatever the resolution of current conflict over its nature it is likely that contraction can be regarded as a shift of a dynamic relation between long and short molecules in the direction of a greater proportion of short ones. A third state of super extension also occurs on stretching. Such a conception informs Eberstein & Stacey's (81) interpretation of their measurements of optical anisotropy of longitudinal muscle of the dog's ileum. Phase retardation of polarized light showed orientation of molecules during stretch, stress relaxation, and active contraction. With stretch a very large change occurs before appreciable tension increase, and as tension rises phase retardation falls again. The first rise may represent ordering of random elements; when all are oriented tension increases as short molecules lengthen. In active contraction, phase retardation decreases in the same direction as during stress relaxation, indicating that shortening and stress relaxation are not simply opposite processes.

Bülbring (82) and her colleagues have fruitfully applied microelectrode techniques to smooth muscle of the guinea pig's taenia coli. At *in situ* length membrane potential is about 60 mv., and it declines to about 43 mv. on stretching to maximum length. Spontaneous spiking occurs at near 1 per sec. with partial depolarization up to 25 per cent of membrane potential. In later reports overshoot is described (83) but it cannot be concluded that as techniques improve an invariable overshoot is revealed. It is not absolutely certain that the microelectrodes are in contact with the cell interior, so the relation between membrane potential and spike cannot yet be dogmatically described. Stimulating drugs cause slow depolarization with increased spike frequency. Each spike is preceded by depolarization of variable slope which, however, is not equivalent to the cardiac pacemaker prepotential, for it can sometimes be completely dissociated from the following spike and be falling before the spike begins. Maintenance of tension is tetanic, for each spike is followed by a small increase in tension with total tension resulting from easy summation (82).

The membrane potential is inversely related to external potassium concentration, but the slope is only 26 to 33 mv. per tenfold concentration change when sulfate is substituted for chloride, and removing calcium depolarizes by 13 mv. (84). In potassium-free solutions spontaneous spiking stops, while with increased potassium spike frequency increases as membrane potential

declines. Then spikes may be converted into oscillations at about 200 per sec. With increased sodium there is an increased spike frequency, but reduction of sodium to 17 mM does not depress frequency of discharge. In general, tension remains proportional to spike frequency so long as spike shape is normal. There is increased potassium permeability during activity, for when the preparation is previously loaded with ^{42}K there is increased outflow of the isotope during spontaneous or induced increase in electrical or mechanical activity (85). There are no data on membrane conductance, and published, as distinct from grapevine, knowledge of electrolyte distribution in intestinal smooth muscle has not improved since Manery (86) found it fragmentary. The recent data of Daniel & Dawkins (87) show high total sodium and chloride as compared with heart and psoas muscle, but there are no independent space measurements from which distribution can be calculated. Rumors of satisfactory observations report high intracellular sodium and chloride. Therefore, neither the membrane potential nor the spike can be interpreted in terms of that fashionable paradigm, the squid giant axone, which as Manery points out, itself has an exceptional electrolyte distribution. The link between excitation and contraction remains mysterious. It can work in the reverse direction, for stretch increases oxygen uptake, potassium efflux, and rate of spiking (82). Metabolic inhibitors may dissociate the two, and in presence of DNP histamine and acetylcholine cause normal increases in spike frequency with no increase in tension (88). Nerve-free smooth muscle of cat's small intestine contracts in response to stimulating drugs although it is completely depolarized by potassium chloride (89).

Nerve-free smooth muscle structures have conducted excitation and coordinated activity (90, 91). One electron-micrographic study (80) shows that the cells are distinctly and completely separated by a cytolemma, but this is directly contradicted by another (92) in which six out of 100,000 sections of ureteral smooth muscle showed bridges joining cells in a manner similar to that of cardiac muscle, forming a functional but not protoplasmic syncytium. The bridges are 2 to 3μ long and 0.3 to 0.5μ in diameter, and their discoverer calculates that each cell must share several bridges with its neighbors. Conduction in the long direction of nerve-free cat's longitudinal intestinal muscle is at 4 cm. per sec., and spontaneous electrical bursts last 0.05 to 0.1 sec., implying simultaneous activity of about 100 fibers. Lateral decrementing conduction occurs at 3 cm. per sec., and an active band spirals around a ring of muscle. This activity can jump a saline bridge or a cut, giving strong support to the idea that fiber to fiber conduction is ephaptic (90). The action potential of one cell does affect its neighbors, for if microelectrodes impale two cells 1 mm. apart the spike in one is frequently associated with small graded potentials in the other (93). Measurements made with the high resistance gap technique show that the impedance of the fiber membrane is not low and that there is a capacitative component (84). The peristaltic reflex, unaffected by destruction of extrinsic innervation, is abolished by interference with the intrinsic plexuses. Schofield [quoted in (95)] describes two types of enteric neurones: (a) multipolar neurones, chiefly in Auerbach's plexus, processes

of which can be traced to muscle coats and are presumably motor, and (b) unipolar or bipolar neurones, chiefly in Meissner's plexus processes of which are distributed on one side to the mucosa and on the other to the first kind of neurones. These latter are presumably sensory. A rise in intraluminal pressure, or appropriate chemical action within the mucosa, stimulates mucosal receptors which, discharging through at least one synapse, elicit the peristaltic reflex (94). The reflex disappears on asphyxiation, anesthetization, or destruction of the mucosa and it is prevented by ganglionic blocking agents. The locus at which drugs are applied profoundly affects their action. Perfusion of the mucosal side by very low concentrations of 5-hydroxytryptamine (5-HT) lowers the intraluminal pressure required to elicit the reflex and itself stimulates peristalsis whereas, applied to the serosal surface, 5-HT inhibits rather than stimulates (95). 5-HT is steadily released by active gut strips, a correlation existing between amount released, intraluminal pressure, and volume of fluid transported. Addition of its precursor, 5-hydroxytryptophan, increases both the amount of 5-HT released and the peristaltic activity. The release continues after ganglionic blockade; and Bülbring concludes that 5-HT, formed and stored within the mucosa, is released in proportion to the rise in intraluminal pressure and that it sensitizes mucosa pressure receptors. This view may be too simple, for pharmacological analysis reveals at least two kinds of 5-HT receptors in the guinea pig's ileum (96, 97).

COORDINATION OF DIGESTIVE FUNCTIONS

Nervous control.—The importance of autonomic reflexes in modifying gut activity varies with species. In sheep and goats coordinated reflex reticuloluminal movements survive decerebration but are abolished by anesthesia (98). Iggo (99) found tension receptors in their gastric wall whose afferent signal evoked vagal discharges. Since the receptors are in series with muscular elements, reflex contraction would be self-perpetuating were it not for a pattern imposed by the center. Uninterrupted afferent stimulation elicits contractions with no less than 10 sec. between them, while similar efferent stimulation produces uncoordinated movements. This reminds one of the swallowing center which emits a stereotyped discharge (100). Paintal (101) described afferent vagal fibers whose receptors appear to lie in the muscularis mucosae of the small intestine. Although the receptors are stimulated by phenyldiguanidine and 513 mM (!) NaCl, they are mechanical receptors. The fibers have a characteristic discharge frequency with peaks 10 to 13 sec. apart, and their activity increases with peristalsis and is correlated with contractions of the muscularis mucosae. A third type of mechanoreceptors is those in the outer layers of the stomach wall which are stimulated by stretch but not by gastric contractions. Finally, there are true chemoreceptors, for Iggo (99a) has isolated 19 afferent vagal fibers whose slowly adapting receptors, scattered without pattern through the gastric mucosa, are sensitive to pH below three or above eight.

The cord pathways and some of the higher projections of gut afferents

have been traced (102); by such a mechanism, distention of the rectum can inhibit spinal monosynaptic reflexes (103). The use of electrical stimulation to trace pathways excited by afferent splanchnic or vagal fibers conceals modalities subserved. Single motor units in the posterior ventral nucleus of the cat's thalamus are excited both by splanchnic stimulation and by stimulation of the appropriate part of the body surface, indicating convergence of pathways below the thalamus (104). These observations are relevant to the problem of referred pain but we need to know whether the thalamic activity also elicits or modifies efferent discharges to the gut. There is a large and growing literature on the effects of stimulation of various parts of the brain upon gastrointestinal function. Unfortunately, gastroenterological experience of investigators is often far below their neurophysiological ability, and the resulting papers [e.g. (105)] have only anecdotal value. Systematic attacks on this important problem by men who are both good gastroenterologists and adequate neurophysiologists have not yet reached the stage of publication. More complete and substantial knowledge may exist in Russia, for the journals in translation now generously supplied by the United States Government afford us a glimpse of what appears to be a vast literature on gastroenterological neurophysiology. The value of the literature is difficult to assess from the fragments seen. In the realm of afferents from the gut, chemoreceptors sensitive to irrigation by peptones and NaHCO_3 or to intravenous nicotine or acetylcholine are described. The reflexes evoked are remarkable. Perfusion of the stomach with peptone increases while perfusion with acid decreases rate of urine formation (106). Stimulation of gastric mechanoreceptors in cats by blowing a balloon to 30 to 40 mm. Hg causes reflex erythropoiesis so that red cell count of peripheral blood rises from 7.57 to 11.7×10^6 in 2 hr. (107).

Bosma's review (108) of the pharyngeal stage of deglutition covers all that is known of the normal and pathological physiology of this complex act. An electromyographic study of the intrinsic laryngeal muscles in man during, among other things, swallowing cannot be summarized (109). At least five groups of workers have applied modern manometric methods to the esophagus with essentially the same results (110 to 115). In man at rest, sphincters are formed by bands of elevated pressure at the pharyngeoesophageal and esophageal-gastric junctions. The upper band is maintained by tonic contraction of striated muscles, its peak pressure averages 26 mm. Hg, and it falls off to about 5 mm. Hg within 2 cm. either way. The esophageal pressure is intrathoracic and therefore less than gastric. The inferior band, whose motor apparatus and innervation are still obscure, is about 1 to 4 cm. long, and its pressure exceeds the gradient of pressure from stomach to esophagus by about 5 mm. Hg. It is usually at but is not identical with the hiatus. When swallowing begins, the elevated pressure of the superior sphincter decreases promptly and briefly to rise again to a high level as the sequence of muscular contractions described by Doty & Bosma (116) occurs. The peristaltic wave engages the esophagus, perhaps reinforced by reflexes driven by slowly

adapting stretch receptors in the cervical esophagus (117). The resting pressure at the inferior sphincter declines with swallowing, rises again to a high level for many seconds, and finally declines to the resting value. Reflex relaxation of the lower sphincter can also be produced by esophageal distention (118). In achalasia the resting pressure of the inferior sphincter is the same as in health, but during swallowing the normal decrease in pressure is absent or of lesser magnitude whereas the subsequent rise in pressure which is the final component of the reflex occurs earlier (119). In diffuse spasm, relaxation of both sphincters and peristalsis in the upper esophagus is normal, but in the lower part the normal wave is replaced by a widespread and prolonged rise in pressure which is exaggerated by volume stimulation (120).

The authoritative review of gastric emptying by Thomas (121) concludes that

... emptying of a meal begins as soon as any considerable part of the gastric contents becomes fluid enough to pass the pylorus. Once started it proceeds rhythmically, a small amount being evacuated once in about 20 seconds in man. Regulation of emptying begins as soon as the evacuated material has accumulated in the intestine to the point where any one of numerous stimuli associated with the chyme reaches threshold value. These stimuli are comprised in the volume and chemical composition of the chyme. The chemical stimuli include the following (listed in order of inhibitory potency when in concentrations usually encountered): fats, fatty acids, proteoses, peptones, amino acids, sugars and other products of starch digestion and hydrogen ions [duodenal pH assumed to lie between 3.5 and 6.0]; also active are osmotically active substances and nonspecific irritants. The effect of these stimuli is to decrease the tone and peristaltic activity of the stomach, thus reducing the pressure gradient which develops with each gastric cycle and which is responsible for the passage of fluid through the pylorus.

In keeping with the conclusion that

the pyloric sphincter plays a part by preventing regurgitation of duodenal contents. . . . It also contracts rhythmically in such a way as, apparently, to limit the volume evacuated at each cycle; but there is as yet no evidence that this function is utilized in regulating the over-all rate of emptying,

Atkinson *et al.* (110) have found *pour épater les radiologistes* that in man there is no zone of high pressure at the pyloric sphincter separating the pressures of the stomach and the duodenum.

Hormonal control.—Gastrin from the antral mucosa stimulates secretion by glands of the corpus, release being effected by distention and protein digestion products. Gastrin release has a nervous component, for application of local anesthetics abolishes response both to chemical and mechanical stimuli (122). A hybrid antrum constructed of colonic mucosa and antral muscle does not secrete gastrin, but one made of antral mucosa and submucosa plus colonic muscle responds to irrigation with alcohol and liver extract with gastrin release but not to distention (123). The effectiveness of vagal impulses in releasing gastrin has been disputed, for denervated corpus pouches usually do not respond to insulin hypoglycemia although vagal

innervation of the antrum remains intact. Dragstedt and co-workers (124) prepared vagally innervated antral pouches separated from the corpus by a mucosal diaphragm. The daily acid output of corpus test pouch was the same as before exclusion of antrum from the digestive tube in contrast with profound reduction occurring when the antrum was excised. When the animals were fed, secretion lasted two to three times longer than before operation. In two such animals insulin hypoglycemia always caused test pouch secretion and in two more it occasionally did so. Because antral mucosa was separated from the corpus, gastrin release caused by vagally induced motility was not masked by the inhibitory effect of the mucosa's being bathed by acid. Another report (125) confirms the conclusion that antral hypermotility caused by insulin hypoglycemia is sufficient to release gastrin. Blood from the antrum passes through the liver before reaching the corpus, and there gastrin may be destroyed. By-passing the liver by means of a portacaval shunt greatly increases response of a dog's denervated pouch to a test meal (126). Gregory (127) occluded the portal vein by taping and found likewise a big increase in the response of a denervated test pouch secretion closely following secretion by the main stomach. He concluded that vagal excitation releases a gastric secretagogue into gastric venous blood.

Contact of acid chyme with antral mucosa reduces or abolishes secretion by a denervated test pouch, and this may result either from interruption of gastrin release or by initiation of release of an inhibitory hormone. Workers in Dragstedt's laboratory (128) prepared two antral pouches, one of which was stimulated by liver extract causing gastrin-mediated secretion in a denervated test pouch. When the other antral pouch was irrigated with acid there was no inhibition and the workers concluded that there is no inhibitory hormone. The experiments in which Jordan & Sand (129), using essentially the same preparation but arousing gastrin release by irrigating one antral pouch with ethanol solution, found inhibition after a long latent period are open to the criticism of Dragstedt (128) that ethanol by virtue of its absorbability is a poor choice of stimulant. However, later experiments by Jordan & Sand (129a) and by Woodward *et al.* (130) demonstrating inhibition of histamine-stimulated secretion by irrigation of an antral pouch with acid indicate inhibition of secretion rather than mere reduction of gastrin release although the latent period of 2 to 4 hr. is a puzzle. Both cephalic and intestinal phases of gastric secretion can also be inhibited by perfusing an antral pouch with acid (131, 132). Is this inhibition mediated by Wolf's (42) inhibitor? A number of thoroughly confusing observations (133, 134, 135) on the effect of surgical manipulation of the antrum would become clearer if this were so.

Duodenal control of gastric secretion (and motility) operates through multiple pathways, and workers in Dragstedt's laboratory (136) have shown that one is by a duodenal hormone at present indistinguishable from secretin which inhibits antral release of gastrin. Gastrin-mediated secretion by a test pouch is abruptly abolished by acid in the duodenum or by injection

of secretin, and inhibition of gastric secretion is closely paralleled by stimulation of pancreatic secretion. There is in addition a positive inhibitory effect, for Sircus (137) found that in irrigating the duodenum with acid, hypertonic NaCl solutions, sugar solutions ranging up to seven times isotonic, peptone, and partially digested fats, all inhibit response to histamine though, to judge by the published figures, inhibition in some instances was dubiously small. There are two separate inhibitory processes. One responding to acid (pH below 2.5) inhibits innervated but not denervated pouches. Since the antrum is not necessary for this, it differs from interference with gastrin release. The other responds to, among others, solutions below 275 mOM and above 425 mOM, and it may be humoral since it affects denervated pouches responding to histamine. The most effective stimulus was the brutal one of perfusing the duodenum with 2200 mOsm. (!) glucose for 30 min.

The problem of the nature of gastrin and the role of histamine in stimulating acid secretion involves two questions. (a) Is histamine an obligatory intermediary for any mode of stimulation? (b) Is gastrin itself histamine? At present, the answer to the first is, probable but not proven. Lane & the Ivys (138) showed that histamine does stimulate acid secretion in the rat, and, since it also stimulates in the mouse, the objection that species exist which do not respond to histamine is weakened. Antihistaminics applied topically in high concentrations to the dog mucosa inhibit acid secretion stimulated either by histamine or urecholine, but Janowitz & Hollander (139) believe it impossible to distinguish among the antihistaminic, atropine-like, and local anesthetic properties of the drugs. The metabolism of histamine is associated with secretion. The glandular part of the rat's stomach contains histidine decarboxylase in concentration ten times that of rabbit's kidney (140). When rats are fed ^{14}C -histidine and simultaneously given a diamine oxidase inhibitor, feeding increases their urinary excretion somewhat less than twofold and decreases the residual amount in the stomach by about half (141). The diamine oxidase inhibitor given to rats or dogs reduces destruction of exogenous histamine and increases acid secretion in dogs given a small test meal or exogenous histamine (142, 143). This evidence, though implicating histamine as a part of natural activation of acid secretion, does not distinguish between histamine as a primary humor (gastrin) from the antrum or as a later intermediary from the corpus. Although Ivy found enough histamine in his own preparations of gastrin to account for their activity, histamine-free gastrins, protein in nature, have been repeatedly prepared by others. However, to settle the problem we need, first, to know the locus of histamine released during acid secretion and, second, to trace completely the course of gastrin by methods thoroughly familiar to endocrinologists. Should a nonhistamine gastrin turn out to contain an easily identifiable tag such as an unusual trace element, it should be relatively easy to satisfy the endocrinological criteria.

Confusion on the role of adrenal cortical hormones in gastric secretion has been confounded by (a) the difficulty of distinguishing among the stimu-

lating, permissive, supportive, and trophic functions of the hormones, (b) the fact that many experiments have been poorly controlled or inadequately reported, and (c) the admixture of extraneous issues such as the significance of stress or hormones in causing or exacerbating ulcers. To assess the action of the hormones in man, Hirschowitz and co-workers (144) measured the effect of 8 hr. infusions of ACTH or adrenal cortical steroids on gastric secretion in cupidious medical students. In 49 experiments on 16 subjects no changes in secretion, except in one instance when aldosterone was used, could be attributed to the hormones. In contrast with these results is the interpretation given by Stempien *et al.* (145) to observations on duodenal ulcer patients without and with complete vagotomy. Insulin hypoglycemia in the first group was followed by gastric secretion sustained 4 hr. or more. In 22 out of 34 tests on vagotomized subjects, hypoglycemia while having no effect in the first 2 hr. was followed in the second two by small amounts, the maximum effect in those called "positive" being about 5 m.eq. as compared with 37 m.eq. in unoperated subjects. This group's earlier work (146, 147) on monkeys indicates that a delayed rise in acidity is associated with stimulation of the pituitary-adrenal axis, although they relied only on pH measurements made on fluid drawn by tube from an unknown locus in an unstated number of observations on animals whose experimental situation must largely be surmised. Nevertheless, the effect of hypoglycemia might be mediated by a different mechanism, for it contains a powerful inhibitory component which survives sympathectomy and bilateral adrenalectomy but not vagotomy (148) and, therefore, vagotomy removes an inhibitory pathway as well as an excitatory one.

The cell population of the gastrointestinal mucosa is in a dynamic state, its status at any time being the resultant of rate of new formation versus rate of destruction. Therefore, health of the mucosa can be determined by changes in any of four factors, those raising or lowering the two separate rates. When cell growth is followed by radioautographic methods after administration of ^{35}S -methionine or ^{14}C -adenine, new cells of the small intestine are seen to be formed in the crypts and to migrate up the villi to the tips where they are lost (149, 150). In rats, starvation for five days decreases the epithelial population one-fifth to one-third as new formation falls behind loss (151). Plasma protein depletion to one-half normal by plasmaphoresis was found to be associated with pyloric and duodenal ulceration in 10 of 21 dogs (152). Adrenal hormones have been accused of promoting ulcers. Janowitz and co-workers (153) found that ACTH or cortisone did not interfere with replacement of mucus epithelium in the dog's stomach after chemical injury, but healing of excision wounds was retarded. They think and others (154) agree the effect of the hormones is on wound healing rather than secretion. Nervous influences are important. Massive acute bloody diarrhea occurred in four of five dogs given acetylbetamethylcholine chloride (mecholyl chloride) following reduction of their red cell and colonic cholinesterase below 30 per cent of normal by a destructive anticholinesterase given

over many weeks (155). Shorter treatment with similar anticholinesterase increases gastric secretion in the dog (156). In attempts to trace higher nervous pathways, French and co-workers (157) found that in 19 monkeys with implanted electrodes in the low middle axis of the hypothalamus "adequate" stimulation over 30 to 80 days was followed by focal lesions in three, duodenal ulcers in three, and diffuse gastric changes in two. Moving still higher, Weiner and co-workers (158) studied 2073 young draft inductees before and after 16 weeks basic training. They measured plasma pepsinogen (which they equated with gastric secretion), response to Rorschach figures, and the occurrence of gastrointestinal lesions. All nine persons developing peptic ulcers were in the upper 15 per cent of the blood pepsinogen distribution and by independent psychological evaluation showed evidence of conflicts about dependency on oral gratification. They conclude that neither high rate of secretion (meaning high plasma pepsinogen) nor specific psychodynamic constitution is responsible for ulceration but the two together are essential determinants on exposure to a noxious social situation.

CONCLUSION

At the end of this survey it would be entertaining and instructive to lunch at the Tour d'Argent and to follow the meal through the gastrointestinal tract, observing in particular how many of the processes actually occurring agree with predictions based on received knowledge. The best we can do is to summarize the work of Borgström (159) who has brought together a variety of new techniques to give for the first time a comprehensive description of digestion and absorption in man. The transintestinal intubation method was used and a standard meal was placed in the stomach. The meal and its transformation were then followed by samples taken at progressively lower levels until it was completely absorbed. By means of tags added to the meal changes in volume, additions and subtraction of components could be followed quantitatively. The meal was 500 ml. of a homogenized mixture of 37 gms. corn oil, 58 gms. skim milk powder, 69 gms. glucose, 0.5 gm. polyethyleneglycol, 0.5 gm. iodinated human serum albumin and water to volume. Its viscosity was not given, and its osmotic pressure must have exceeded 770 mOsm. The meal was not lobster and champagne, but it was better than, say, 2 per cent pectin plus 3.5 per cent sucrose with a dash of phenol red. It was delivered to the duodenum from the stomach over 4 hr., the largest portion (35 per cent) in the second hour. Emptying was not logarithmic. The pH of the gastric contents declined from about 4-5 during the first hour to around 2 during the fourth hour, but the pH of the duodenal contents was 6 and increased to 7 distally. It was never below 5. This is higher than expected, and it raises questions about what reflexes and hormones were stimulated in the duodenum. As the meal passed the duodenum it was diluted three to five times. Glucose was diluted to about 280 mOsm. and was seldom over isotonic concentration. Pancreatic enzymes were highest in duodenal contents and in the proximal jejunum, but they re-

mained fairly high throughout the small intestine. The major locus of intestinal enzyme action was intracellular, confirming again the established fact that enzymes are present in the gut contents as the result of desquamation, not secretion. The gall bladder emptied in the first 30 min., and a total of 4 to 8 gms. of bile mixed with the chyme. Since the total bile acid pool is 2 to 4 gms. (160), enterohepatic circulation occurred twice. Absorption began in the duodenum and was complete in the first 100 cm. of jejunum, being more proximal for fat than for carbohydrate which in turn was absorbed more proximally than protein. Protein was absorbed 80 to 90 per cent, glucose and lactose 100 per cent, and fat which was not significantly diluted by endogenous fat 90 to 95 per cent. It is clear that this work gives only a fragmentary answer to the problems outlined in the first paragraph of this review, but it is also clear that it represents an important thrust forward. It, together with much else discussed here, is as good in its own way as work in any other branch of physiology and there is no reason to be discouraged about the state of gastroenterological physiology.

LITERATURE CITED

1. Burgen, A. S. V., *J. Cellular Comp. Physiol.*, **45**, 465-78 (1955); **48**, 113-38 (1956)
- 1a. Burgen, A. S. V., *J. Physiol. (London)*, **132**, 20-39 (1956)
- 1b. Burgen, A. S. V., *Can. J. Biochem. and Physiol.*, **36**, 409-11 (1958)
2. Burgen, A. S. V., and Seeman, P., *Can. J. Biochem. and Physiol.*, **35**, 481-89 (1957); **36**, 119-43 (1958)
3. Pappenheimer, J. R., *Physiol. Revs.*, **33**, 387-423 (1953)
4. Logothetopoulos, J. H., and Myant, N. B., *J. Physiol. (London)*, **134**, 189-94 (1956)
5. Lundberg, A., *Physiol. Revs.*, **38**, 21-40 (1958)
- 5a. Lundberg, A., *Acta Physiol. Scand.*, **35**, 1-25 (1955); **40**, 21-34 (1957); **40**, 35-58 (1957); **40**, 101-12 (1957)
6. Denton, D. A., *Quart. J. Exptl. Physiol.*, **42**, 72-95 (1956)
- 6a. Denton, D. A., *J. Physiol. (London)*, **131**, 516-25 (1956); **135**, 227-44 (1957); **140**, 129-47 (1958)
7. Coats, D. A., and Wright, R. D., *J. Physiol. (London)*, **135**, 611-22 (1957)
8. Denton, D. A., and McDonald, I. R., *J. Physiol. (London)*, **138**, 44-62 (1957)
9. McDonald, I. R., and Denton, D. A., *Nature*, **177**, 1035-36 (1956)
10. Goding, J. R., and Denton, D. A., *Australian J. Exptl. Biol. Med. Sci.*, **35**, 301-20 (1957)
11. Langley, L. L., Gunthorpe, C. H., and Beall, W. A., *Am. J. Physiol.*, **192**, 482-84 (1958)
12. Chauncey, H. H., and Weiss, P. A., *Arch. intern. pharmacodynamie*, **113**, 377-83 (1958)
13. Chauncey, H. H., Lisanti, V. F., and Winer, R. A., *Proc. Soc. Exptl. Biol. Med.*, **97**, 539-42 (1958)
14. Kostlin, A., and Rauch, S., *Helv. Med. Acta*, **24**, 600-21 (1957)
15. Shannon, I. L., and Prigmore, J. R., *Proc. Soc. Exptl. Biol. Med.*, **97**, 825-28 (1958)
16. Guth, P. H., Komarov, S. A., Shay, H., and Style, C. Z., *Am. J. Physiol.*, **187**, 207-23 (1956); **192**, 1-13 (1958)
17. Buchs, S., *Z. physiol. Chem.*, **301**, 201-9 (1955)
18. Richmond, V., Tang, J., Wolf, S., Caputto, R., and Trucco, R. E., *Federation Proc.*, **17**, 297 (1958)
19. Miller, L. L., Segal, H. L., and Plumb, E. J., *Gastroenterology*, **33**, 566-74 (1957)
20. Hirschowitz, B. I., *Physiol. Revs.*, **37**, 475-511 (1957)
21. Friedman, E., Poliner, I. J., and Spiro, H. M., *New Engl. J. Med.*, **257**, 901-5 (1957)
22. Poliner, I. J., and Spiro, H. M., *Gastroenterology*, **34**, 196-209 (1958)
23. Spiro, H. M., Ryan, A. E., and Jones, C. M., *Gastroenterology*, **30**, 563-82 (1956)
24. Earle, A. S., and Hoar, C. S., Jr., *Surgery*, **43**, 583-94 (1958)
25. Jones, G. M., *Quart. J. Exptl. Physiol.*, **42**, 390-97 (1957)
26. Gryboski, W. A., and Spiro, H. M., *New Engl. J. Med.*, **255**, 1131-34 (1956)
27. Kowalewski, K., *Can. J. Biochem. and Physiol.*, **35**, 729-32 (1957)
28. Hirschowitz, B. I., and Streeten, D. H. P., *J. Lab. Clin. Med.*, **50**, 209-15 (1957)
29. Goidsenhoven, G. van, Wilkoff, L., and Kirsner, J. B., *Gastroenterology*, **34**, 421-35 (1958)

30. Segal, H. L., Miller, L. L., Reichsman, F., Plumb, E. J., and Glaser, G. L., *Gastroenterology*, **33**, 557-65 (1957)
31. Halminen, E., *Gastroenterologia*, **89**, 93-100 (1958)
32. Murphy, Q. R., Ed., *Metabolic Aspects of Transport Across Cell Membranes*, 277-346 (University of Wisconsin Press, Madison, Wis., 379 pp., 1957)
33. Heniz, E., and Durbin, R. P., *J. Gen. Physiol.*, **41**, 101-17 (1957)
34. Durbin, R. P., and Heniz, E., *J. Gen. Physiol.*, **41**, 1035-47 (1958)
35. Janowitz, H. D., Dreiling, D. A., Roblin, H. L., and Hollander, F., *Gastroenterology*, **33**, 378-84 (1957)
36. Dennis, W. H., and Rehm, W. S., *Am. J. Physiol.*, **193**, 15-20 (1958)
37. Rehm, W. S., Dennis, W. H., and Brodsky, W. A., *Am. J. Physiol.*, **192**, 14-22 (1958)
38. Yakhnina, D. N., *Biokimiya*, **21**, 429-33 (1956) [English Transl., **21**, 433-37 (1956)]
39. Yakushiji, T., Kikuci, T., Yamamoto, J., and Kuriaki, K., *Am. J. Physiol.*, **192**, 476-78 (1958)
40. Glass, G. B. J., Rich, M., and Stephanson, L., *Gastroenterology*, **34**, 598-615 (1958)
41. Ellenbogen, L., Burson, S. L., and Williams, W. L., *Proc. Soc. Exptl. Biol. Med.*, **97**, 760-64 (1958)
42. Smith, W. O., Hoke, R., Landy, J., Caputto, R., and Wolf, S., *Gastroenterology*, **34**, 181-87 (1958)
43. Pasternak, C. A., Kent, P. W., and Davies, R. E., *Biochem. J.*, **68**, 212-17 (1958)
44. Pasternak, C. A., and Kent, P. W., *Biochem. J.*, **68**, 452-57 (1958)
45. Zipkin, I., Adamik, E. R., and Saroff, H. A., *Proc. Soc. Exptl. Biol. Med.*, **95**, 69-71 (1957)
46. Patton, J. R., and Pigman, W., *Science*, **125**, 1292-93 (1957)
47. Laird, A. K., and Barton, A. D., *Biochim. et Biophys. Acta*, **25**, 58-62 (1957)
48. Siekevitz, P., and Palade, G. E., *J. Biophys. Biochem. Cytol.*, **4**, 203-17 (1958)
49. Nickerson, J. F., Kraus, F. W., and Perry, W. I., *Proc. Soc. Exptl. Biol. Med.*, **95**, 405-8 (1957)
50. Cohen, H., Megel, H., and Kleinberg, W., *Proc. Soc. Exptl. Biol. Med.*, **97**, 8-10 (1958)
51. Weil, L., and Telka, M., *Arch. Biochem. Biophys.*, **71**, 204-20 (1957)
52. Sarda, L., Marchis-Mouren, G., Constantin, M. J., and Desnuelle, P., *Biochim. et Biophys. Acta*, **23**, 264-74 (1957)
53. Junqueira, L. C. U., Rothschild, H. A., and Fajer, A., *Exptl. Cell Research*, **12**, 338-41 (1957)
54. Schneyer, L. H., and Schneyer, C. A., *Am. J. Physiol.*, **193**, 1-3 (1958)
55. Dickman, S. R., and Morrill, G. A., *Am. J. Physiol.*, **190**, 403-7 (1957)
56. Craddock, D. M., and Dalglish, C. E., *Biochem. J.*, **66**, 250-55 (1957)
57. Hong, S. S., and Magee, D. F., *Am. J. Physiol.*, **191**, 71-74 (1957)
58. Magee, D. F., and White, T. T., *Am. J. Physiol.*, **193**, 21-24 (1958)
59. Visscher, M. B., Varco, R. H., Carr, C. W., Dean, R. B., and Erickson, D., *Am. J. Physiol.*, **141**, 488-505 (1944)
60. Visscher, M. B., Fetcher, E., Carr, C. W., Gregor, H., Bushey, M., and Barker, D., *Am. J. Physiol.*, **142**, 550-75 (1944)
61. Grim, E., Lee, J., and Visscher, M. B., *Am. J. Physiol.*, **182**, 359-63 (1955)

62. Fisher, R. B., and Parsons, D. S., *J. Physiol.*, **110**, 36-46 (1949)
63. Lifson, N., and Parsons, D. S., *Proc. Soc. Exptl. Biol. Med.*, **95**, 532-34 (1957)
64. Smyth, D. H., and Taylor, C. B., *J. Physiol. (London)*, **136**, 632-48 (1957); **141**, 73-80 (1958)
65. Riklis, E., and Quastel, J. H., *Can. J. Biochem. and Physiol.*, **36**, 347-62 (1958); **36**, 363-71 (1958)
66. Riklis, E., Haber, B., and Quastel, J. H., *Can. J. Biochem. and Physiol.*, **36**, 373-80 (1958)
67. Papadopoulos, N. M., and Roe, J. H., *Am. J. Physiol.*, **189**, 301-6 (1957)
68. Kiyasu, J. Y., and Chaikoff, I. L., *J. Biol. Chem.*, **224**, 935-39 (1957)
69. Borgström, B., Tryding, N., and Westöö, G., *Acta Physiol. Scand.*, **40**, 241-47 (1957)
70. Linder, E., and Blomstrand, R., *Proc. Soc. Exptl. Biol. Med.*, **97**, 653-57 (1958)
71. Tidwell, H. C., *Proc. Soc. Exptl. Biol. Med.*, **98**, 12-16 (1958)
72. Neame, K. D., and Wiseman, G., *J. Physiol. (London)*, **140**, 148-55 (1958)
73. Wiseman, G., *J. Physiol. (London)*, **136**, 203-07 (1957)
74. Hird, F. J. R., and Sidhu, G. S., *Biochim. et Biophys. Acta*, **25**, 388-93 (1957)
75. Lluch, M., *Riv. Españ. fisiol.*, **13**, 107-13 (1957)
76. Annegers, J. H., *Am. J. Physiol.*, **191**, 75-80 (1957)
77. Tidball, C. S., and Tidball, M. E., *Am. J. Physiol.*, **193**, 25-28 (1958)
78. Simmonds, W. J., *Quart. J. Exptl. Physiol.*, **42**, 205-21 (1957)
79. Hanson, J., and Lowy, J., *Nature*, **180**, 906-09 (1957)
80. Caesar, R., Edwards, G. A., and Ruska, H., *J. Biophys. Biochem. Cytol.*, **3**, 867-77 (1957)
81. Eberstein, A., and Stacey, R. W., *Am. J. Physiol.*, **192**, 290-96 (1958)
82. Bülbring, E., *J. Physiol. (London)*, **122**, 111-34 (1953); **125**, 302-15 (1954); **135**, 412-25 (1957)
83. Holman, M. E., *J. Physiol. (London)*, **136**, 569-84 (1957); **137**, 77-78P (1957)
84. Burnstock, G., and Straub, G. W., *J. Physiol. (London)*, **140**, 156-67 (1958)
85. Born, G. V. R., and Bülbring, E., *J. Physiol. (London)*, **131**, 690-703 (1956)
86. Manery, J. F., *Physiol. Revs.*, **34**, 334-418 (1954)
87. Daniel, E. E., and Dawkins, O., *Am. J. Physiol.*, **190**, 71-76 (1957)
88. Bülbring, E., and Lullmann, H., *J. Physiol. (London)*, **136**, 310-23 (1957)
89. Evans, D. H. L., and Schield, H. O., *Nature*, **180**, 341-42 (1957)
90. Prosser, C. L., and Rafferty, N. S., *Am. J. Physiol.*, **187**, 546-48 (1956)
91. Prosser, C. L., and Sperelakis, N., *Am. J. Physiol.*, **187**, 536-45 (1956)
92. Bergman, R. A., *Bull. Johns Hopkins Hosp.*, **102**, 195-202 (1958)
93. Bülbring, E., Burnstock, G., and Holman, M. E., *J. Physiol. (London)*, **140**, 52-53P (1958)
94. Bülbring, E., Lin, R. C. Y., and Schofield, G., *Quart. J. Exptl. Physiol.*, **43**, 26-37 (1958)
95. Bülbring, E., and Lin, R. C. Y., *J. Physiol. (London)*, **140**, 381-407 (1958)
96. Kosterlitz, H. W., and Robinson, J. A., *J. Physiol. (London)*, **136**, 249-62 (1957)
97. Gaddum, J. H., and Picarelli, Z. P., *Brit. J. Pharmacol.*, **12**, 323-28 (1957)
98. Titchen, D. A., *J. Physiol. (London)*, **141**, 1-21 (1958)
99. Iggo, A., *J. Physiol. (London)*, **131**, 248-56 (1956)
- 99a. Iggo, A., *Quart. J. Exptl. Physiol.*, **42**, 398-409 (1957)
100. Doty, R. W., *Am. J. Physiol.*, **166**, 142-58 (1951)
101. Paintal, A. S., *J. Physiol. (London)*, **139**, 353-68 (1957)

102. Downman, C. B. B., and Evans, M. H., *J. Physiol. (London)*, **137**, 66-79 (1957)
103. Evans, M. H., and McPherson, A., *J. Physiol. (London)*, **140**, 201-12 (1958)
104. McLeod, J. G., *J. Physiol. (London)*, **140**, 462-78 (1958)
105. Shealy, C. N., and Peele, T. L., *J. Neurophysiol.*, **20**, 135-39 (1957)
106. Kuchinskii, E. P., *Bull. Exptl. Biol. Med., U.S.S.R.*, **41**, 195-97 (1956) in English transl.
107. Kan. E. L., *Bull. Exptl. Biol. Med., U.S.S.R.*, **41**, 381-85 (1956) in English transl.
108. Bosma, J. F., *Physiol. Revs.*, **37**, 275-300 (1957)
109. Faaborg-Andersen, K., *Acta Physiol. Scand.*, **41**, Suppl. 140, 1-149 (1957)
110. Atkinson, M., Edwards, D. A., Honour, A. J., and Rowlands, E. N., *Lancet*, **II**, 918-22 (1957)
111. Fleshler, B., Hendrix, T. R., Kramer, P., and Ingelfinger, F. J., *J. Appl. Physiol.*, **12**, 339-42 (1958)
112. Muller Botha, G. S., Astley, R., and Carré, I. J., *Lancet*, **I**, 659-62 (1957)
113. Schlegel, J. F., and Code, C. F., *Am. J. Physiol.*, **193**, 9-14 (1958)
114. Texter, E. C., Jr., Smith, H. W., Moeller, H. C., and Barborka, C. J., *Gastroenterology*, **32**, 1013-24 (1957)
115. Smith, H. W., Texter, E. C., Jr., Stickley, J. H., and Barborka, C. J., *Gastroenterology*, **32**, 1025-47 (1957)
116. Doty, R. W., and Bosma, J. F., *J. Neurophysiol.*, **19**, 44-60 (1956)
117. Andrews, B. L., *J. Physiol. (London)*, **134**, 729-40 (1956)
118. Creamer, B., and Schlegel, J., *J. Appl. Physiol.*, **10**, 498-504 (1957)
119. Creamer, B., Olsen, A. M., and Code, C. F., *Gastroenterology*, **33**, 293-301 (1957)
120. Creamer, B., Donoghue, F. E., and Code, C. F., *Gastroenterology*, **34**, 782-96 (1958)
121. Thomas, J. E., *Physiol. Revs.*, **37**, 453-74 (1957)
122. Woodward, E. R., and Shapiro, H., *Am. J. Physiol.*, **192**, 479-81 (1958)
123. Baugh, C. M., Bravo, J. L., Barcena, J., and Dragstedt, L. R., *Surg. Forum*, **7**, 356-60 (1957)
- 123a. Baugh, C. M., Bravo, J. L., Barcena, J., and Dragstedt, L. R., *Arch. Surg.*, **76**, 441-46 (1958)
124. Oberhelman, H. A., Rigler, S. P., and Dragstedt, L. R., *Am. J. Physiol.* **190**, 391-95 (1957)
125. Woodward, E. R., Robertson, C., Fried, W., and Shapiro, H., *Gastroenterology*, **32**, 868-77 (1957)
126. Clarke, J. S., Hart, J. C., and Ozeran, R. S., *Proc. Soc. Exptl. Biol. Med.*, **97**, 119-21 (1958)
127. Gregory, R. A., *J. Physiol. (London)*, **137**, 76-77P (1957)
128. Longhi, E. H., Greenlee, H. B., Bravo, J. L., Guerrero, J. D., and Dragstedt, L. R., *Am. J. Physiol.*, **191**, 64-70 (1957)
129. Jordan, P. H., Jr., and Sand, B. F., *Proc. Soc. Exptl. Biol. Med.*, **94**, 471-74 (1957)
- 129a. Jordan, P. H., Jr., and Sand, B. F., *Surgery*, **42**, 40-49 (1957)
130. Woodward, E. R., Trumbull, W. E., Shapiro, H., and Towne, L., *Am. J. Digest. Diseases*, **3**, 204-19 (1958)
131. Black, J. W., Fisher, E. W., and Smith, A. N., *J. Physiol. (London)*, **141**, 22-26 (1958)

132. State, D., and Morgenstein, L., *Surg., Gynecol. Obstet.*, **106**, 545-48 (1958)
133. Andersson, S., Elwin, C. E., and Uvnas, B., *Gastroenterology*, **34**, 636-58 (1958)
134. Jones, T. W., Stevenson, J. K., Jesseph, J. E., Nyhus, L. M., and Harkins, H. N., *Ann. Surgery*, **147**, 13-16 (1958)
135. Margolus, B. D., and Harrison, R. C., *Surg. Forum*, **7**, 360-65 (1957)
136. Greenlee, H. B., Longhi, E. H., Guerrero, J. D., Nelsen, T. S., El-Bedri, A. L., and Dragstedt, L. R., *Am. J. Physiol.*, **190**, 396-402 (1957)
137. Circus, W., *Quart. J. Exptl. Physiol.*, **43**, 114-33, (1957)
138. Lane, A., Ivy, A. C., and Ivy, E. K., *Am. J. Physiol.*, **190**, 221-28 (1957)
139. Janowitz, H. D., and Hollander, F., *Proc. Soc. Exptl. Biol. Med.*, **95**, 320-25 (1957)
140. Schayer, R. W., *Am. J. Physiol.*, **189**, 533-36 (1957)
141. Schayer, R. W., and Ivy, A. C., *Am. J. Physiol.*, **189**, 369-72 (1957); **193**, 400-2 (1958)
142. Ivy, A. C., Lin, T. M., Ivy, E. K., and Karvinen, E., *Am. J. Physiol.*, **186**, 239-44 (1956)
143. Lin, T. M., Ivy, A. C., Karvinen, E., and Ivy, E. K., *Am. J. Physiol.*, **186**, 231-38 (1956)
144. Hirschowitz, B. I., Streeten, D. H. P., London, J. A., and Pollard, H. M., *J. Clin. Invest.*, **36**, 1171-82 (1957)
145. Stempien, S. J., French, J. D., Dagradi, A., Movius, H. J., II, and Porter, R. W., *Gastroenterology*, **34**, 104-10 (1958); **34**, 111-16 (1958)
146. Porter, R. W., Movius, H. J., and French, J. D., *Surgery*, **33**, 875-80 (1953)
147. French, J. D., Longmire, R. L., Porter, R. W., and Movius, H. J., *Surgery*, **34**, 621-32 (1953)
148. Geziri, M. F., Robertson, C., Plzak, L. F., and Woodward, E. R., *Surgery*, **43**, 606-9 (1958)
149. Leblond, C. P., Everett, N. B., and Simmons, B., *Am. J. Anat.*, **101**, 225-56 (1958)
150. Walker, B. E., *Anat. Record*, **127**, 383 (1957)
151. Stevens Hooper, C., and Blair, N., *Exptl. Cell Research*, **14**, 175-81 (1958)
152. Hahn, P. F., Baugh, P., and Foster, D. L., *Proc. Soc. Exptl. Biol. Med.*, **95**, 238-41 (1957)
153. Janowitz, H. D., Weinstein, V. A., Shaer, R. G., Cerghini, F., and Hollander, F., *Gastroenterology*, **34**, 11-20 (1958)
154. Drye, J. C., and Schoen, A. M., *Ann. Surgery*, **147**, 738-48 (1958)
155. Sleisinger, M. H., Lewis, C. M., Pert, J. H., Roseman, D. R., Nickel, W. F., Jr., and Almy, T. P., *Gastroenterology*, **34**, 582-97 (1958)
156. Telléz-Gerón, E., and Villarreal, R., *Gastroenterology*, **34**, 874-78 (1958)
157. French, J. D., Porter, R. W., Cavanaugh, E. B., and Longmire, R. L., *Psychosomat. Med.*, **19**, 209-20 (1957)
158. Weiner, H., Thaler, M., Reiser, M. F., and Mirsky, I. A., *Psychosomat. Med.*, **19**, 1-10 (1957)
159. Borgström, B., Dahlqvist, A., Lundh, G., and Sjövall, J., *J. Clin. Invest.*, **36**, 1521-36 (1957)
160. Lindstedt, S., *Acta Physiol. Scand.*, **40**, 1-9 (1957)
161. De Wardener, H. E., and Herxheimer, A., *J. Physiol. (London)*, **139**, 53-63 (1957)
162. Dreisbach, R. H., *Proc. Soc. Exptl. Biol. Med.*, **96**, 555-58 (1957)

163. Ferguson, M. H., Naimark, A., and Hildes, J. A., *Can. J. Biochem. and Physiol.*, **35**, 333-37 (1957)
164. Kitahara, S., *Kumamoto Med. J.*, **11**, 12-17 (1958)
165. Lane, A., Ivy, A. C., and Ivy, E. K., *Am. J. Physiol.*, **191**, 262-64 (1957)
166. Leonsins, A. J., and Waddell, W. R., *J. Appl. Physiol.*, **12**, 334-38 (1958)
167. Lin, T. M., and Alphin, R. S., *Am. J. Physiol.*, **192**, 23-26 (1958)
168. Ray, F. E., and Pease, P., *Am. J. Physiol.*, **190**, 109-12 (1957)
169. Richmond, V., Caputto, R., and Wolf, S., *Arch. Biochem. Biophys.*, **66**, 155-66 (1957)
170. Robinson, R. M., Harris, K., Hlad, C. J., and Eisman, B., *Proc. Soc. Exptl. Biol. Med.*, **96**, 518-20 (1957)
171. Schneyer, C. A., *Proc. Soc. Exptl. Biol. Med.*, **98**, 160-64 (1958)
172. Sun, D. H. C., and Shay, H., *J. Appl. Physiol.*, **11**, 148-54 (1957)
173. Heatley, N. G., *Nature*, **181**, 1069-70 (1958)
174. Lin, T. M., and Ivy, A. C., *Am. J. Physiol.*, **189**, 361-68 (1957)
175. Menguy, R. B., Hallenbeck, G. A., Bollman, J. L., and Grindlay, J. H., *Surg., Gynecol. Obstet.*, **106**, 306-20 (1958)
176. Necheles, H., *Am. J. Physiol.*, **191**, 595-97 (1957)
177. Tinkel, H. I., and Hollander, F., *Am. J. Physiol.*, **193**, 393-99 (1958)
178. Borgström, B., *Acta Chem. Scand.*, **11**, 749 (1957)
179. Eriksson, S., *Proc. Soc. Exptl. Biol. Med.*, **94**, 578-82 (1957)
180. Gilleland, J. L., Gast, J. H., and Halpert, B., *Proc. Soc. Exptl. Biol. Med.*, **94**, 118-19 (1957)
181. Grim, E., and Smith, G. A., *Am. J. Physiol.*, **191**, 555-60 (1957)
182. Yonehiro, E. G., Imamoglu, K., Root, H. D., Crisp, N. W., Wangenstein, S. L., and Wangenstein, O. H., *Proc. Soc. Exptl. Biol. Med.*, **97**, 249-51 (1958)
183. Bangham, D. R., and Terry, R. J., *Biochem. J.*, **66**, 579-83 (1957)
184. Byers, S. O., and Friedman, M., *Am. J. Physiol.*, **191**, 87-89 (1957)
185. Deutsch, H. F., and Smith, V. R., *Am. J. Physiol.*, **191**, 271-76 (1957)
186. Duffy, B. J., Jr., and Turner, D. A., *Ann. Internal Med.*, **48**, 1-7 (1958)
187. Geiger, E., Human, L. E., and Middleton, M. J., *Proc. Soc. Exptl. Biol. Med.*, **97**, 232-34 (1958)
188. Grossman, M. I., and Jordan, P. H., Jr., *Gastroenterology*, **34**, 892-900 (1958)
189. Hogben, C. A. M., Schanker, L. S., Tocco, D. J., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, **120**, 540-45 (1957)
190. Hyde, A. S., *Am. J. Physiol.*, **191**, 265-70 (1957)
191. Kaplan, E., Edidin, B. D., Fruin, R. C., and Baker, L. A., *Gastroenterology*, **34**, 901-09 (1958)
192. Rendina, G., Sarcione, E. J., Lee, C. J., and Barrett, H. W., *Proc. Soc. Exptl. Biol. Med.*, **95**, 350-53 (1957)
193. Sandweiss, D. J., and Levy, S. H., *Proc. Soc. Exptl. Biol. Med.*, **95**, 259-61 (1957)
194. Schanker, L. S., Shore, P. A., Brodie, B. B., and Hogben, C. A. M., *J. Pharmacol. Exptl. Therap.*, **120**, 528-39 (1957)
195. Schwartz, R. D., Cohn, G. L., Bondy, P. K., Brodoff, M., Upton, G. V., and Spiro, H. M., *Proc. Soc. Exptl. Biol. Med.*, **97**, 648-50 (1958)
196. Tidwell, H. C., *Am. J. Physiol.*, **189**, 537-41 (1957)
197. Tidwell, H. C., and Johnston, J. M., *Proc. Soc. Exptl. Biol. Med.*, **94**, 150-51 (1957)

198. Vassar, P. S., and Taylor, D. M., *Proc. Soc. Exptl. Biol. Med.*, **93**, 504-6 (1956)
199. Zawoiski, E. J., Baer, J. E., Braunschweig, L. W., Paulson, S. F., Shermer, A., and Beyer, K. H., *J. Pharmacol. Exptl. Therap.*, **122**, 442-48 (1958)
200. Byers, S. O., and Friedman, M., *Am. J. Physiol.*, **192**, 427-31 (1958)
201. Glover, J., and Green, C., *Biochem. J.*, **67**, 308-16 (1957)
202. Ivy, A. C., Karvinen, E., Lin, T. M., and Ivy, E. K., *J. Appl. Physiol.*, **11**, 1-7 (1957)
203. Ivy, A. C., Suzuki, R., and Prasad, C. R., *Am. J. Physiol.*, **193**, 521-24 (1958)
204. Karvinen, E., Lin, T. M., and Ivy, A. C., *J. Appl. Physiol.*, **11**, 8-11 (1957); **11**, 143-47 (1957)
205. Karvinen, E., Lin, T. M., and Ivy, A. C., *Gastroenterology*, **33**, 789-93 (1957)
206. Lin, T. M., Karvinen, E., and Ivy, A. C., *Am. J. Physiol.*, **190**, 214-20 (1957)
207. Smith, A. L., Hauk, R., and Treadwell, C. R., *Am. J. Physiol.*, **193**, 34-40 (1958)
208. Swell, L., Trout, E. C., Jr., Hopper, J. R., Field, H., Jr., and Treadwell, C. R., *Proc. Soc. Exptl. Biol. Med.*, **98**, 174-76 (1958)
209. Vahouny, G. V., and Treadwell, C. R., *Am. J. Physiol.*, **191**, 179-84 (1957)
210. Vahouny, G. V., Woo, C. H., and Treadwell, C. R., *Am. J. Physiol.*, **193**, 41-46 (1958)
211. Anderson, B., and Wyrwicka, W., *Acta Physiol. Scand.*, **41**, 194-98 (1957)
212. Andersson, B., and Jewell, P. A., *J. Physiol. (London)*, **139**, 191-97 (1957)
213. Cohen, M. J., Landgren, S., Strom, L. and Zotterman, Y., *Acta Physiol. Scand.*, **40**, Suppl. 135, 1-50 (1957)
214. Gilbert, G. J., *Anat. Record*, **126**, 253-66 (1956)
215. Gilbert, G. J., *Am. J. Physiol.*, **191**, 243-47 (1957)
216. Morrison, S. D., Barnett, R. J., and Mayer, J., *Am. J. Physiol.*, **193**, 230-34 (1958)
217. Morrison, S. D., and Mayer, J., *Am. J. Physiol.*, **191**, 248-54 (1957); **191**, 255-61 (1957)
218. Ikeda, M., Hayama, T., Chujyo, N., and Hoshi, A., *Am. J. Physiol.*, **192**, 27-29 (1958)
219. Innes, I. R., Kosterlitz, H. W., and Robinson, J. A., *J. Physiol.*, **137**, 396-409 (1957)
220. Kiessling, A., *Z. Biol.*, **109**, 336-49 (1957)
221. Flood, C. A., Colcher, H., and Mathers, J., *Gastroenterology*, **34**, 410-20 (1958)
222. Hughes, F. B., *Am. J. Physiol.*, **191**, 37-39 (1957)
223. Sparchez, T., and Stoichita, S., *Gastroenterologia*, **89**, 1-18 (1958)
224. Lepkovsky, S., Lyman, R., Fleming, D., Nagumo, M., and Dimick, M. M., *Am. J. Physiol.*, **188**, 327-31 (1957)
225. Schofield, B., *Gastroenterology*, **33**, 714-29 (1957)
226. Schulman, J. L., Carleton, J. L., Whitney, G., and Whitehorn, J. C., *J. Appl. Physiol.*, **11**, 419-21 (1957)
227. Stubbe, J. L., *Gastroenterology*, **33**, 693-702 (1957)
228. Waddell, W. R., *J. Appl. Physiol.*, **12**, 468-72 (1958)
229. Black, J. W., Fisher, E. W., and Smith, A. N., *J. Physiol. (London)*, **141**, 27-34 (1958)
230. Fletcher, T. L., Dahl, A. W., Jesseph, J. E., Steinbock, H. L., and Harkins, H. N., *Proc. Soc. Exptl. Biol. Med.*, **95**, 559-60 (1957)
231. Haverback, B. J., and Bogdanski, D. F., *Proc. Soc. Exptl. Biol. Med.*, **95**, 392-93, (1957)

232. Haverback, B. J., Bogdanski, D., and Hogben, C. A. M., *Gastroenterology*, **34**, 188-95 (1958)
233. Haverback, B. J., Hogben, C. A. M., Moran, N. C., and Terry, L. L., *Gastroenterology*, **32**, 1058-65 (1957)
234. Kasich, A. M., and Argyros, T. G., *Gastroenterology*, **34**, 232-38 (1958)
235. Krogsgaard, A. R., *Acta Med. Scand.*, **158**, 1-11 (1957)
236. Nordgren, B., and Obrink, K. J., *Acta Physiol. Scand.*, **40**, 297-304 (1957)
237. Rider, J. A., Moeller, H. C., and Gibbs, J. O., *Gastroenterology*, **33**, 737-44 (1957)
238. Smith, A. N., Black, J. W., and Fisher, E. W., *Nature*, **180**, 1127 (1957)
239. Smith, M. J. H., *Am. J. Physiol.*, **193**, 29-33 (1958)
240. Toh, C. C., *J. Physiol. (London)*, **38**, 488-94 (1957)
241. Udenfriend, S., and Weissbach, H., *Proc. Soc. Exptl. Biol. Med.*, **97**, 748-51 (1958)
242. Watt, J., and Wilson, C. W. M., *J. Physiol. (London)*, **137**, 61-62P (1957)
243. Loran, M. R., and Althausen, T. L., *Am. J. Physiol.*, **193**, 516-20 (1958)
244. Moog, F., and Thomas, E. R., *Physiol. Zool.*, **30**, 281-87 (1957)
245. Necheles, H., Gordon, M. R., Gaspar, H., and Walker, L., *Am. J. Physiol.*, **190**, 252-54 (1957)
246. Robert, A., and Nezamis, J. E., *Proc. Soc. Exptl. Biol. Med.*, **98**, 9-12 (1958)
247. Shay, H., Sun, D. C. H., Dlin, B., and Weiss, E., *J. Appl. Physiol.*, **12**, 461-67 (1958)
248. Skoryna, S. C., Webster, D. R., and Kahn, D. S., *Gastroenterology*, **34**, 1-10 (1958)
249. Jávora, T., and Varró, V., *Gastroenterology*, **32**, 119-25 (1957)
250. Bean, J. W., and Sidky, M. M., *Am. J. Physiol.*, **189**, 541-47 (1957)
251. Brauer, R. W., Holloway, R. J., and Leong, G. F., *Am. J. Physiol.*, **189**, 24-30 (1957)
252. Davis, W. D., Jr., Batson, H. M., Jr., Reichman, S., Gorlin, R., and Storaasli, J. P., *Gastroenterology*, **34**, 52-64 (1958)
253. Farrand, E. A., Larsen, R., and Horvath, S. M., *Am. J. Physiol.*, **189**, 576-79 (1957)
254. Horvath, S. M., Kelly, T., Folk, G. E., Jr., and Hutt, B. K., *Am. J. Physiol.*, **189**, 573-75 (1957)
255. Selkurt, E. E., *Am. J. Physiol.*, **193**, 599-604 (1958)
256. Sidky, M., and Bean, J. W., *Am. J. Physiol.*, **193**, 386-92 (1958)
257. Börgstrom, B., *Scand. J. Clin. & Lab. Invest.*, **9**, 226-28 (1957)
258. Burge, H., and Vane, J. R., *Brit. Med. J.*, **I**, 615-18 (1957)
259. Komarov, S. A., and Marks, I. N., *Proc. Soc. Exptl. Biol. Med.*, **97**, 574-75 (1958)
260. Love, J. W., *Quart. J. Exptl. Physiol.*, **42**, 279-84 (1957)
261. Lundh, G., *Scand. J. Clin. & Lab. Invest.*, **9**, 229-32 (1957)
262. MacCannon, D. M., and Horvath, S. M., *Am. J. Physiol.*, **189**, 569-72 (1957)
263. Reynell, P. C., and Spray, G. H., *Gastroenterology*, **34**, 867-73 (1958)

HEART¹

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ANATOMY

A detailed model of the arrangement of structural filaments in striated muscle has been presented by Huxley (1), who advances ingenious and convincing arguments for it. Undoubtedly cardiac muscle is similar in basic ultrastructure. Muir (2) and Sjöstrand and co-workers (3) have described the ultrastructure of the intercalated discs of several species. These discs are double-membrane continuations of the cellular membrane, and thus divide the muscle into discrete units. The membranes are extensively folded and may not be serious barriers to electrical current; this may account for the fact that macroscopically cardiac muscle appears to be a functional syncytium. Moore & Ruska (4) have also observed the fine structure of a cardiac muscle. Fawcett & Selby (5) have described the ultrastructure of the turtle atrium. This tissue lacks the cytoplasmic reticulum found in other myocardium, which some have considered to conduct electrical activity from the membrane to the inside of the muscle.

The numbers and condition of muscle and supporting fibers in the normal human heart and that with valvular disease have been described by Schoenmackers (6). Oken (7) quantitated collagen in the human myocardium. Thomas (8) analyzed in detail the muscular architecture of hog and dog ventricles, and Northup and co-workers (9) related age, sex, and body size to the ratio of heart to body weight in dogs. The cardiovascular anatomy of a fetal giraffe was described by Keen & Goetz (10). Sharma (11) has reported the cardiac anatomy and function of *Rana tigrina*.

James & Burch (12) studied the blood supply of the human interventricular septum and human atria (13). Halpern (14) reports that the rat heart has both coronary and extracoronary blood vessels, the latter possibly a retention from the fishes. Intercoronary anastomoses in several species were measured by Vastesaegeer and co-workers (15). The hog normally has few intercoronary anastomoses, and the number increases greatly after chronic experimental coronary occlusion [Paul *et al.* (16)].

Prakash studied the conduction system of the krait (17) and the spiral valve of the tadpole (18) which apparently serves as a conduction system from the bulbus cordis to the truncus arteriosus. Bhatnagar (19) described conduction tissue in the lizard. Marked changes in the conduction system in a human diabetic were reported by Köberle (20). Gresham (21) described networks of fine (possibly Purkinje) fibers crossing the right heart in man.

Using anatomical and physiological techniques in the dog, Coleridge and co-workers (22) found atrial receptors to be located in the atriocaval

¹ The survey of the literature pertaining to this review was concluded June, 1958.

junction, the pulmonary veins, and left atrium. Afferent vagal impulses in the frog are about 80 per cent from the lung and 17 per cent from the heart according to Kolatatz *et al.* (23). The distribution of receptors was given in detail. White (24) described the anatomy and mechanisms of cardiac pain. Studies carried out by Waites in the sheep (25) and by Kato and co-workers in rats (26) and rabbits (27) showed species differences in the anatomical arrangement of the inhibitory and accelerator efferent pathways.

METABOLISM

The heart is an excellent subject for metabolic studies; unlike other tissues, it does not contain proteolytic enzymes which destroy other enzymes before they can be extracted. The studies directed at fundamental biochemical properties, far beyond this reviewer's competence, will not be covered in this review. The 1957 review by Lorber (28) should be consulted by interested readers.

According to Duffey & Ebert (29) the metabolism of heart-forming areas in the chick embryo depends on oxidative pathways for DPN. Heart and brain, therefore, differ in their electron transport systems. In the absence of anterior pituitary or insulin, cardiac glycogen may parallel blood glucose, according to Lukens (30). Heart tissue, therefore, may not need insulin for glucose transport. Fawaz *et al.* (31) found that malonate in concentrations which block the Krebs cycle in muscle-mince did not affect performance or high-energy phosphate content of the canine heart-lung preparation.

Hollander & Webb (32) found that adenine compounds shorten the duration of depolarization in the rat atrium. On the other hand, Fleckenstein and collaborators (33) found that ATP restored the duration of the action potential and the magnitude of the resting potential when both had been depressed by increased potassium, and Macfarlane (34) obtained similar results. Grauer (35) studied the effects of anoxia with and without hypocalcemia on ATP levels in the rat heart. Creatine phosphate partially prevented the fall in ATP produced by thyroxine, while creatine did not [Angelucci *et al.* (36)]. Nucleotide levels in human atrial biopsy material were measured by Burdette (37).

Some aspects of phosphorus metabolism and effects of the vagus on these have been studied by Raiskina (38). In perfused rat hearts, Hess & Haugaard (39) found that aminophylline and epinephrine increase phosphorylase activity, indicating conversion of the enzyme from an inactive to an active form. Hearn & Wainio (40) noted increased aldolase activity in rat hearts after exercise. Slowing of conduction, decreased rate of impulse formation, and other electrical changes resulted from perfusion of isolated guinea pig heart with glucose-free solutions [Penna *et al.* (41)]. Glucose also potentiates potassium asystole [Jordan *et al.* (42)]. Picard (43) studied the relationship between iron-binding and lipid-containing granules.

Van Citters *et al.* (44) found a basal oxygen consumption in the canine Langendorff preparation of 1.98 cc. per 100 gm. per min. and a contractile metabolism (without external work) of 0.83 to 1.18 cc. oxygen per 100 gm.

per 100 beats. External work at a slower heart rate thus requires lower energy. Sarnoff and co-workers (45) described a nonfailing, metabolically supported, perfused heart preparation in which metabolism and function could be studied. No significant oxygen debt was incurred by the preparation. The total tension developed by the heart was the primary determinant of oxygen consumption (46). The critical oxygen saturation of coronary sinus blood is 6 to 7 per cent (the pressure being 4 to 5 mm. Hg) [Bretschneider *et al.* (47)]. At lower figures glycolysis and failure ensue, hypertrophied hearts being more failure-prone. Berglund and co-workers (48) found oxygen consumption generally below 3.5 ml. per min. in the arrested heart. During fibrillation the consumption was four to nine times as high. However, in other studies only a slight difference in oxygen consumption was found between the arrested, fibrillating, or empty contracting heart [Beuren *et al.* (49)].

Myocardial oxygen consumption is unchanged with vagal stimulation or acetylcholine [Schreiner *et al.* (50)]. Whalen (51) reported a new technique for simultaneous recording of oxygen consumption and contraction of small muscles. In the 72-hr. chick, Paff & Boucek (52) found alterations in rate and rhythm with hypoxia (12 and 18 per cent oxygen). Lower oxygen produced irreversible arrhythmia or asystole (reversible by 4 per cent carbon dioxide). Nahas & Cavert (53) produced heart failure with carbon dioxide over 5 per cent. Epinephrine, norepinephrine, or acetyl strophanthidin reversed the failure.

Niedergerke (54) has hypothesized that calcium facilitates contraction by acting at the cell surface, since tension changes preceded movement of the ion into the cells. Merrick (55) found that tissue slices synthesized more acid-soluble glycogen in an electrolyte-free medium than when in a solution containing up to 0.1 osmolar sodium and 0.025 osmolar potassium. Further increase in either ion induced glycogenolysis or depressed synthesis. Oxygen consumption was also measured. Johnson (56) observed a sodium flux of 15×10^{-12} M per cm^2 per sec. across the frog ventricular membrane. Caviezel *et al.* (57) found that potassium lack depresses calcium transport, possibly by raising the intracellular sodium concentration. Beznak (58) reported that cholinesterases have a positive inotropic effect and act with membrane calcium to regulate purine metabolism.

ELECTROPHYSIOLOGY

Cellular electrophysiology.—An exhaustive review of this subject has been prepared by Crane-field & Hoffman (59), and the entire field was widely covered in a conference [Hecht (60)]. Weidmann's monograph on cardiac electrophysiology has been translated into Japanese (61), and Weidmann (62) has briefly reviewed the ionic changes in heart muscle. Shanes (63) has reviewed the physiological and pharmacological aspects of the subject at length. Also of importance to all who deal with cardiac electrical activity is a symposium on active transport (64) which includes data both on heart cells and on transport systems in other tissues.

At present it appears that the resting potential is maintained by a sodium-potassium pump, that the initial rapid phase of depolarization results from rapid inward movement of sodium ions as a result of increased permeability to sodium, that the plateau is the result of decreased permeability to potassium and a low, but greater than diastolic, sodium permeability, and that repolarization is a regenerative return to the resting state. The evidence for this is well covered in the reviews (59, 60, 62, 63) in the various articles, and in the conference [Weidmann (65); Brady & Woodbury (66); Hecht (67)].

Wilde's (68) work demonstrates an efflux of potassium during systole. Differences between the action potentials of the various excitable tissues probably stem from differences in the sequence of the permeability changes during electrical systole [Tasaki & Hagiwara (77)]. Aside from the sodium permeability changes during the initial phase of depolarization and the potassium efflux during systole, our knowledge of permeability changes and ionic fluxes in cardiac muscle is largely a matter of indirect evidence and hypothesis. An ideal experimental object to further knowledge of cellular electrical events would be a large, slowly depolarizing, cardiac cell with an extracellular space that can be instantaneously altered in composition so that during part of a single action potential the outside medium can be changed and sequential alterations in ion permeability thus followed throughout a cycle. In some of Weidmann's earlier experiments he conducted studies of this type with potassium as far as techniques would permit.

Intracellular potentials have been recorded from different parts of the heart in several mammals [Coraboeuf *et al.* (69)]; from the perfused frog heart [Ware *et al.* (70)], and from the dog fish [Gargoul & Coraboeuf (71)]. Hoffman and co-workers (72) reported that the intracellular potentials of the atrioventricular node resemble those from pacemaker cells. Intracellular potentials recorded from the human ventricle during surgery are identical with those seen in other species [Woodbury *et al.* (73)].

Carmeliet & Lacquet (74) find that the shortening of the intracellular action potential caused by rapid stimulation is increased by high extracellular potassium and low extracellular or high intracellular sodium. They attribute the frequency effects to the variations in sodium and potassium. Trautwein & Dudel (75) have demonstrated that acetylcholine induces a specific increase of potassium permeability in the atrium. Marshall (76) reported that low temperature decreased the resting and action potentials of rabbit atria. Pacemaker cells were most resistant. When propagation of impulses had been abolished it could be re-established by acetylcholine. Tasaki & Hagiwara (77) used tetraethylammonium chloride to alter the action potential of the squid axon so that it became similar to that of cardiac muscle. Although they consider their results evidence that the membrane has two distinct stable states, the depolarized state of their altered axon, like that of cardiac muscle, is relatively unstable. After administration of epinephrine, Otsuka (78) observed increased steepness of potentials and increases in both the resting potential and the depolarization potential of cardiac cells, probably caused by epinephrine increasing the activity of the sodium pump.

Otsuka & Gürtner (79) found that vitamin-B₁ deficiency did not alter resting or active potentials of the rat myocardium. Thiamine apparently does not directly influence the basic ion-pumping mechanisms.

Some relationships between biochemical and electrical events were briefly summarized by Macfarlane (34). He postulates that factors which reduce action potential duration, such as increased stimulation frequency, interfere with high-energy phosphate stores, which normally cause active inward ion movement during the depolarization plateau. The experimental results obtained by Fleckenstein *et al.* (33) and by Hollander & Webb (32) with ATP are also pertinent. Crane-field & Hoffman (80) have demonstrated that electrically induced repolarization may be propagated through a fiber, and that with low calcium, repolarization can propagate over longer distances. They do not, however, believe that repolarization is normally propagated in the ventricle.

Excitability.—Crane-field and co-workers (81) confirm previous reports that the threshold is lower for anodal than for cathodal excitation during most of the relative refractory period; during the early relative refractory period, only the anode can excite. Using a papillary muscle-Purkinje fiber preparation, Hoffman *et al.* (82) studied refractoriness. Both a local response and a change in local action potential are important to the changes in conduction velocity resulting from stimulation during the refractory period. Ullrick & Sommers (83) found human trabecular heart tissue perfused post mortem to be revivable and contractile, and similar in these properties to other cardiac tissue in many cases. Time from death to perfusion was important in determining revivability. In experiments by Laforet *et al.* (84), pentobarbital induced small increases in refractory period without changes in excitability. Mendez & Mendez (85) found cardiac glycosides to cause a displacement (elevation) of the strength-duration curves and slowing of conduction which were greater in the atria than in the ventricle.

Pathways of excitation.—The first records of activity within the atrioventricular node have been published in two reports, one based on use of intracellular electrodes [Hoffman *et al.* (72)] and the other on the use of extracellular electrodes [Scher *et al.* (86)]. The first of these studies includes evidence concerning the nature of cellular activity in the region, and both studies indicate that slow conduction rather than a latency accounts for what is loosely called "atrioventricular nodal delay". Deutsch (87) studied atrioventricular conduction in the turtle at varying rates of atrial stimulation.

Several papers by Burchell (88), Scher & Young (89), Durrer & van der Tweel (90), and Medrano *et al.* (91) in the conference cited above have covered recent findings concerning ventricular activation. The first areas to be depolarized are at the septal termination of the left and right bundles. Mural excitation is generally from within outward with more rapid movement in the innermost layers. The basal left wall and septum are depolarized last. Newer publications by Medrano *et al.* (92, 93, 94) support the previously debated conclusion that the interventricular septum is generally excited from both endocardial surfaces by a wave of excitation proceeding toward the

center. Erickson *et al.* (95) have analyzed the general spread of excitation in bundle-branch block, and Becker *et al.* (96) have plotted the pathway in left bundle-branch block. In bundle-branch block the wave of excitation reaches the blocked ventricle by conduction from the intact side, and there is no area of delay in the septum as has been postulated. Increased duration of the QRS complex is discussed. Frimpter *et al.* (97) analyzed complete bundle-branch block in man vectorcardiographically and found that the pathway of excitation is similar to that which has been described for the dog.

Dubouloz (98) has considered the relationship between recorded potentials and the pathway of excitation in the wall. A paper by Pipberger and co-workers (99) refutes previous conclusions from the same laboratory that the inner portion of the wall is "electrically silent".

Derivation of the electrocardiogram from pathways of excitation seems qualitatively possible although there are some questions [Johnston (100); Scher & Young (89)]. Relationships between ventricular activation and vectorcardiographic loops have been analyzed by Sodi-Pallares *et al.* (101).

Little appears to be known about the repolarization pathways at present. Pipberger *et al.* (102) have presented some experimental data which is followed by discussion of other ideas. The U wave is considered at length in the monograph (60, pp. 942-59) and is even less well understood than the T wave at present. Ferrero (103) finds a correlation between the amplitude of the U wave and the degree of ventricular filling.

Electrocardiography and vectorcardiography.—As usual, many reports concerning conventional electrocardiography have appeared during the past year. These will not be discussed here. Much electrocardiographic research concerns the use of the vectorcardiogram to replace conventional unipolar electrocardiograms partially or fully. The problem can be briefly stated as follows. Does ventricular depolarization produce potentials on the body surface which cannot be distinguished from those which would be produced by three (or fewer) independent and fixed voltage sources within the body? If the answer to this query is affirmative, then three (or fewer) independent electrocardiographic leads will contain all the information which can possibly be recorded and these can be viewed vectorally. If the answer is negative (and if such an answer is more than a quibble), more than three leads must be recorded, and vectoral representation is impossible. This question has been stated in detailed mathematical form by Burger (104), geometrically by Okada (105), and in general terms by Katz (106). Relevant papers by Schmitt (107), Frank (108), and Burch (109) have appeared during the year. Most of the evidence which is now appearing tends to support the vectorcardiographic approach. Wolferth (110) has considered this matter editorially. Johnston (100) has pointed out that even should three leads suffice, these can be recorded in a scalar fashion.

Okada (111) has experimentally studied multiple dipole potentials and the effects of inhomogeneities in volume conductors. Reynolds and co-workers (112) have discussed the use of the lead-field concept in the development of leads for vectorcardiography and described electrode placement for

a sagittal lead. Abildskov (113) has presented a system for displaying vectorcardiograms on a linear scale. Pipberger *et al.* (114) have designed an electronic resolver which attempts to answer the question whether there is more information in multiple leads than in the vectorcardiogram. Helm has devised a system for scalar presentation of orthogonal leads (115) and a circuit for three-plane stereovectorcardiography (116). Whipple (117) has criticized multiple leads and proposed that vectorcardiographic recording replace or supplant them for some purposes. His reference to the unipolar versus the dipolar approach confuses the technique (unipolar) with the concept of the nature of the source (dipolar). Sodi-Pallares and co-workers (101) conclude that vectorcardiographic curves give valid information about the sequence of ventricular activation.

Milnor (118) proposes a system of vectorcardiographic classification based on a single plane for the normal QRS loop and deviations from this plane. Langner and co-workers (119) obtained similar vector loops (with some exceptions) from four systems of lead placement. Seiden (120) has described normal vector loops using the Frank system of electrode placement plus an electronic resolver. Duchosal & Moret (121) have defined normal and abnormal vector loops and have found a better anatomicoelectrical axis correlation between these than between anatomical and electrical axes calculated from scalar leads. They consider details of the loop more important than rotation in the early and late phases of depolarization. Simonson (122) has tabulated normal values for several electrocardiographic parameters and listed sources of variability in these.

Kahn & Simonson (123) have studied effects of anerobic exercise on conventional and vectoral electrocardiograms and conclude that general hypoxia rather than localized ischemia occurs. Lamb (124) concludes that respiratory variations in the electrocardiogram depend on changes in right and left ventricular stroke volume in addition to other factors. Simonson *et al.* (125) report many respiratory changes in vectorcardiograms and comment that changing anatomical relationships will not explain the observed changes.

The ventricular gradient has received some notice. This construction represents the difference in area and duration of QRS and T. If, as appears possible, the depolarization and repolarization of the ventricles are relatively independent phenomena, the gradient has no special significance. Brinberg (126) has presented a technique for determination of the gradient, and Gärtner & Schaefer (127) argue that if the gradient concept is useless, the analysis of the T wave is likewise unwarranted. Gardberg & Rosen (128) have presented a strong argument for the use of the gradient and Briller (129) has constructed an apparatus to display it. Cogent arguments regarding the ventricular gradient are to be found on page 905 *et seq.* of the New York Academy monograph (60).

Brody (130) described a tank method for determining the effective vectorcardiogram of *Rana pipiens* and after using this method reported that the tetrahedral system is not isometric. Vectorcardiograms in the normal dog were studied by Horan *et al.* (131). Irisawa & Irisawa (132) have described

the electrocardiogram of the mantis shrimp, which has a neurogenic pacemaker. Schwan & Kay (133, 134) have painstakingly measured the conductivity and capacitance of heart, lung, blood, and other tissues. Bayley has discussed exploratory lead systems and zero potentials (135).

Donoso *et al.* (136) find that application of potassium chloride to the canine ventricles produces abnormal current flows only during diastole. They bravely extrapolate to other types of injury and consider true ST segment shifts reported by others to be experimental artifacts. Alzamora-Castro *et al.* (137) have studied the effects of injury on potentials recorded through a potassium chloride electrode.

Arrhythmias, flutter, and fibrillation.—Since these entities were first recognized, the causes of flutter and fibrillation have been matters for dispute. Lewis' circus movement has been criticized and it has been proposed that either one or many rapidly discharging foci can be involved in these arrhythmias. Some conflict stems from the dubious assumption that electrical and drug-induced arrhythmias are identical and from the belief that the factors which initiate flutter or fibrillation also perpetuate the conditions. Evidence that these assumptions may be erroneous has appeared previously and several of the papers below are relevant.

Kenamer *et al.* (138) believe that flutter resulting from electrical stimulation is similar to aconitine-induced arrhythmias in that a rapidly discharging focus causes the arrhythmia. Their excessively short stimulating pulse (0.02 msec.) makes it likely that severe damage occurred at the stimulus site and that the experiments do not disprove Lewis' conclusions. In disagreement with Kenamer and co-workers, Kimura *et al.* (139) and Kato *et al.* (140) conclude that aconitine-induced flutter and electrically-induced flutter are different. Cooling the site of extrasystoles has led Scherf (141) to the conclusion that some sort of afterdischarge is important in their genesis. It has also been found (142) that carbon dioxide does not alter flutter or fibrillation induced by aconitine, with or without cooling, as it has been said to alter other forms of extrasystoles.

Angelakos & Shepherd (143) found a periodic discharge, which does not persist, in early ventricular fibrillation in hypothermic dogs. However, utilizing microelectrodes, Sano *et al.* (144) observed no repetitive activity in homothermic ventricular fibrillation. Covino & Beavers (145) find that cooling dogs to 22°C. decreases the threshold for ventricular fibrillation fivefold and that prior acclimatization to cold alters this effect (146). Milnor and co-workers (147) found that single capacitor discharges delivered outside of the "vulnerable period" can cause fibrillation. Maling & Moran (148) reported on arrhythmias induced by sympathomimetic amines following coronary occlusion.

Many studies have been concerned with the ionic changes which facilitate, inhibit, or accompany fibrillation. Once induced, fibrillation was more likely to continue in environments low in sodium, potassium, or chloride, according to Kärki (149). Figures are given for percentage of hearts which fibrillate (from 0 to 100) as calcium concentration is held constant and potassium is

varied. Steffenson and co-workers (150) studied the effects of restricting dietary potassium on cyclopropane-epinephrine tachycardia. Susceptibility to arrhythmia was lowered if less potassium had been mobilized. Cherbakoff *et al.* (151) reported that infusions of insulin plus glucose reduce the coronary sinus potassium concentration and the incidence of fibrillation from coronary ligation. The incidence of fibrillation following coronary occlusion was reduced by perfusion of the ischemic area with Ringer-Locke solution containing magnesium [Carden & Steinhaus (152)]. Grumbach (153) defibrillated rabbit hearts with solutions low in sodium. He suggests that these solutions, like procaine or high potassium, depress sodium entrance during the rising phase of repolarization and concludes from this that fibrillation must involve multiple ectopic foci. According to Klein & Holland (154) the normal potassium efflux is 3.45 pmoles per cm^2 per sec. and during fibrillation increases transiently to 9.37, an effect greater than the combined effect of acetylcholine and high-frequency stimulation. Potassium influx rose, then fell; other changes are described. The most unique accompaniment of fibrillation is the potassium efflux. Since the changes are transient, it appears that initiation and maintenance of fibrillation are separate phenomena. Holland & Tinsley (155) suggest that increased outward potassium flux and increased total sodium flux resulting from increased permeability produce fibrillation which is maintained by acetylcholine. Brown & Prasad (156) studied ionic changes which occur during the onset of fibrillation when a very hypercapnic animal is returned to normal carbon dioxide concentrations. As fibrillation occurs under these conditions, the ratio of ultrafilterable calcium to potassium, already low owing to changes in both variables during hypercapnia, falls further to one-third of the control value. Since total calcium is not much affected it is felt that some nonfilterable phosphate is formed.

MECHANICAL EVENTS

Factors affecting rate.—Ringer *et al.* (157) report changes in heart rate of the developing chick associated with age, sex, gonadectomy, and gonadotropin administration. Dilating the sinoauricular chamber of the frog's heart with pressures up to 2 to 6 cm. H_2O increased the rate of impulse formation [Pathak (158)]. Effects of vagal stimulation on the rat heart rate at various temperatures led Mainwood (159) to the theory that each vagal impulse releases or destroys a substance which varies in concentration with heart rate. Wilhelmj and co-workers (160) found that fasting followed by overeating produced increased arterial pressure and heart rate in dogs and that sympathectomy prevented the rise in pressure. Penaz (161) has shown that there are periodic nonrespiratory variations in the cardiac rate in man coincident with variations in vasomotor tone. Lester (162) described a transducer for continuous registration of heart rate.

Factors affecting contractility.—Hajdu *et al.* (163) found that β -palmitoyl lysolecithin, a substance present in blood and mammalian tissues, has a digitalis-like effect on myocardial contractility in several animal preparations. Green & Nahum (164) have tentatively identified palmitoyl lyso-

lecithin as the substance in liver (lecithin) which increases contractility. Tyramine was among several other compounds which altered contractility. A cardioactive substance was found in the spleen by Cobbin & Thorp (165). Cornman (166) has described cardiac stimulation and inhibition (rate and amplitude) by deoxycorticosterone in low and high doses. This inhibition is blocked by serum or serum fraction (167). The potent serum fraction has a molecular weight of 60,000 to 70,000 and requires a dialyzable cofactor. Benson *et al.* (168) found that glycerol-extracted trabecular muscle bundles from failing dog hearts were weaker than those from controls and they concluded that actomyosin is structurally changed in congestive heart failure. A significant diminution in contractility of actomyosin bands prepared from failing human hearts as compared with controls was found by Kako & Bing (169) and could be corrected by digoxin plus calcium chloride. The results support the rarely held idea that digoxin acts directly on the contractile mechanism. Whalen (170) found an apparent exception to the "all-or-none" law in cat papillary muscle, since increased stimulation intensity caused a change in amplitude of contraction in papillary muscle preparations. He used "all-or-none" to refer to contraction, although this term is usually restricted to electrical events, and attributes the increase in contraction to release of epinephrine or a related substance and the inhibition to acetylcholine since the responses appear to be abolished by the proper blocking agents. Gersch & Deuse (171) studied effects of factors inducing coagulation (calcium and vitamin K separately and in combination) on contractility. Bucher (172) has recorded the contraction of cardiac cells in tissue culture photoelectrically.

Cardiac hemodynamics.—Brandt (173) has considered the closing mechanism of the human tricuspid valve in the light of anatomical studies. Pressure-volume relationships in the human left ventricle were estimated by Burch *et al.* (174). Fowler and co-workers (175) reported on the systolic and diastolic relationships in the isolated rat heart. A negative early diastolic pressure developed, the magnitude of which correlated with the vigor of contraction. Brecher & Kissen (176, 177) showed that a negative ventricular diastolic pressure develops when the ventricle is operating at an approximately normal arterial pressure and normal end diastolic volume. Sarnoff and co-workers (45) reported the characteristics of an isolated, perfused heart preparation. This preparation was essentially similar to the heart of the anesthetized dog with open chest.

The influence of stroke volume, rate, aortic pressure, hypothermia, sympathomimetic amines, and valvular regurgitation on duration and rate of ventricular ejection and filling was determined by Braunwald and co-workers (178). Greater stroke volume leads to greater duration and rate of ventricular ejection. Greater heart rate at a constant output leads to shorter duration of ejection per beat and lowers the rate of ejection. Cotten & Maling (179) found a linear relationship between ventricular contractile force and ventricular stroke work when atrial or aortic pressures were altered and when *l*-epinephrine was given. Ouabain in nontoxic doses increases contractility of the nonfailing heart. Other effects on the circulation were described.

Mazzella (180) has noted the effects of distention on the resting and active tension and on the staircase phenomenon in the toad ventricle. Guyton and co-workers (181) reported that epinephrine increases cardiac output "to a far greater extent by the increased tendency for venous return than by the heart's increased pumping ability."

Methods of minimizing hydrostatic sources of error in catheterization of the left heart were discussed by Roy *et al.* (182). Oppenheimer and co-workers (183) produced functional mitral regurgitation by vagal stimulation leading to diastasis in dogs, and described electrokymographic curves, recorded directly over the atrium, which indicate the regurgitant flow. Paul *et al.* (184) used cinefluorography to demonstrate regurgitation through normal mitral valves in dogs.

Attinger (185) studied effects of respiration on cardiac pressure pulses. Dubois & Marshall (186) found pulmonary blood flow constant throughout the respiratory cycle. On inspiration there was greater right heart output and greater vascular resistance. Reduction of left heart output follows as a result of increased pulmonary venous and capillary capacity. Figley (187) has discussed the advantages of adding angiocardiology to electrical and hemodynamic measurements in evaluating the state of the heart.

Heart sounds.—In a symposium on the mechanism of production of heart sounds McKusick (188) reveals the limits of knowledge in this field. In addition to valvular movements, turbulence and cavitation can produce sounds, but statements on the contribution of these to specific sounds or murmurs can still provoke disagreement. The first two sounds are generally held to be of valvular origin. The variations in the amplitude of the first heart sound with varying P-Q interval in dogs with complete heart block were studied by Siecke & Essex (189). Hemodynamic changes were also produced. These workers conclude that atrioventricular valvular opening during atrial diastole is important in these variations. Positive and negative pressure differences across the mitral valve in dogs with complete atrioventricular block were associated with high and low amplitudes of the first heart sound (190). The stated differences accompanied opened and closed atrioventricular valves respectively. Reinhold & Rudhe (191) reaffirm Leatham's concept that the first sound has mitral and tricuspid components and that the second sound has aortic and pulmonary valvular components. Lewis and co-workers (192) have developed a means of high-fidelity heart sound recording using modified sonar techniques. On the right, the first and third sounds are loudest in the right ventricle, the second sound in the pulmonary artery, and the fourth in the atrium. Using the chest wall as one plate of a variable capacitor, Groom & Sihvonen (193) have produced voltages which represent movements of the wall. They feel that this device is well adapted to phonocardiography. Luisada (194) has described techniques for human intracardiac phono- and electrocardiography. The symposium mentioned above is followed by a consideration of clinical aspects (195). Technical standards for phonocardiography have been proposed (196). Interestingly, the aim is to produce voltages proportional to the ear's nonlinear perception of sound rather than to the actual amplitude of the vibration.

CARDIAC OUTPUT

There has been excellent and concerted study of indicator dilution techniques during the year. A series of papers from the Mayo Clinic by Wood and collaborators discusses recording and basic patterns (197), and evaluation of valvular defects [Wood & Woodward (198); Wright & Wood (199)]. The use of a new tricarboyanine dye "Fox Green" eliminates changes in oxygen content of the blood as a source of error (200, 201). Dynamic characteristics of systems used in recording indicator dilution curves have been studied by Fox *et al.* (202) who reported that hydraulic factors rather than transducers were most important in overall performance. A similar conclusion was reached by Lacy *et al.* (203).

Emanuel and co-workers (204) reported a technique for continuous calibration of dilution curves, and Grace and co-workers (205) report a technique for determination of thoracic aorta flow in man by constant injection. Fritts and co-workers (206) have successfully used the pulmonary artery in man as sampling site for the determination of right ventricular output. Revised equations for blood flow and volume calculations are presented by Keys *et al.* (207). Goodwin & Sapirstein (208) have developed the use of autogenous plasma together with a conductivity cell for the determination of cardiac output by hematocrit dilution. Lochner & dal Ri (209) have described a blood dilution technique for measurement of cardiac output, and Booth *et al.* (210) have used such a technique for detection of shunts. Radiopotassium was used by Conn and co-workers (211) as an indicator in evaluating angiocardiology. Hayden and co-workers (212) have used dilution curves to set up electronic analogues for the evaluation of mitral insufficiency. Quantitation of backflow in aortic insufficiency has been described by Warner & Toronto (213). Huff *et al.* (214) report on the detection of intravenously injected tagged human serum albumin by four precordial scintillation counters. This technique is used to measure and calculate cardiac output and other circulatory parameters in humans.

Several studies have concerned the use of the ballistocardiogram and an evaluation of its present status has been presented by Scarborough & Baker (215). The newer instruments are technically much improved but have not been adequately tested. It seems possible that the ballistocardiograph will have clinical value although its use in measuring cardiac output is doubtful. Changes in human cardiac output with exercise were studied with this technique [Klensch & Hohnen (216)] as were effects of respiration [Klensch, Hohnen & Kessler (217)], of altered intrapulmonary pressure [Hohnen & Klensch (218)], and of morphine-barbiturate anesthesia and abdominal compression in dogs [Scarborough (219)]. New ballistocardiographic instruments with wide frequency range have been described by Reeves *et al.* (220) and Frederick & Eddlemen (221), and a specific correlation with cardiovascular events has been suggested (222). Starr & Schild report on the accuracy of two methods for estimating stroke volume from the peripheral pulse (223). Porje & Rudewald (224) derived a new formula for determining stroke volume from a number of physical measurements, as did Evans (225). All of these tech-

niques are better for determining changes in an individual than for comparing individuals.

Brotmacher (226) and Lüthy (227) have concluded that derivation of cardiac output from arterial pressure is not accurate. Starr (228) reports that the area under the pulse wave is related to the stroke volume, the pulse pressure to the work and velocity of injection. The dynamic function of the heart can be best evaluated by considering not only the pulse wave but also the mean pressure and the pulse wave velocity. Suggestions are made regarding the improvement of estimations of stroke volume from the peripheral pulse. Barer & Nüsser (229) find that cardiac output (pulmonary blood flow) fell during forced inflation of the lungs and rose during expiration. Both increased pulmonary vascular resistance and decreased cardiac filling inhibited the decreased flow with positive pressure. Critical closing pressure was between 1 and 12 mm. Hg.

Of interest to those using catheterization are values for oxygen saturation of the chambers provided by Barrat-Boyes & Wood (230) and for left heart pressures provided by Roy *et al.* (182). Weissler and co-workers (231) describe effects on cardiac output of altering the central venous reservoir by postural changes. Factors influencing pressures on the right side of the heart were studied during separate perfusion of the greater and lesser circulations [Weil *et al.* (232)]. Studies of the pulmonary vasculature will not be covered because of space limitations.

CARDIAC CONTROL

As indicated in some of the studies of cardiac hemodynamics, it is still common to analyze cardiac responses to stress in terms of such mechanical properties as are embodied in Starling's law of the heart. The mechanical properties of the heart can be entirely altered by nervous reflexes and it is doubtful that circulating hormones exert potent regulatory effects. The studies below attempt to delineate the nature of the central control of the heart; differences in conclusions may depend on presence or absence of anesthesia among other variables.

Rushmer (233) found changes both in rate and in left ventricular performance on sympathetic stimulation. It was possible partially to cancel these effects by vagal stimulation, but complete cancellation was impossible since the vagus predominantly affected rate while the sympathetics predominantly affected performance. Randall and co-workers (234) have stimulated at many points along the preganglionic pathways followed by the sympathetic motor nerves to the heart. Although the effects on rate and stroke volume were generally not separable between the spinal cord and the heart, preferential effects on rate were seen on the right, and on strength of contraction on the left. Cotten & Moran (235) reflexly augmented sympathetic activity by stimulation of peripheral receptors. The predominant effect was on vasomotor tone, while influences on cardiac contractile force were moderate. Direct stimulation of the cardiac sympathetic nerves or administration of *l*-norepinephrine, however, augmented right ventricular contractile force.

Lovegrove and co-workers (236) found that the increase in cardiac output seen with anemia in dogs was not altered by adrenalectomy (with adequate adrenocortical substitution therapy) or by adrenalectomy plus ganglionic blockade. The sympathoadrenal system was thus not necessary for the adaptation of the cardiac output to the severe anemia. Rohse *et al.* (237) found low frequency stimulation of the stellate ganglion produced slower, greater, and better maintained rise in arterial pressure than high frequency. The effect was attributed to increased contractile force.

Carlsten and co-workers (238) directly stimulated the vagus nerve in patients undergoing surgery of the neck. By blocking descending impulses and stimulating, they estimated the tonic vagal discharge at 2 to 4 impulses per sec. No decrease in stroke volume resulted from vagal stimulation. Koepchen & Thureau (239) found that a Bainbridge reflex (increased heart rate after infusion of saline) was only slightly decreased when the vagus was blocked. This reflex is not effected only through the vagus.

CORONARY BLOOD FLOW

When cardiac work is increased, coronary flow generally increases. Hemodynamic, reflex, and chemical factors possibly responsible for this increase have been widely studied and the last of these is now considered most important. The search for the specific metabolite which increases coronary flow has not been successful.

According to Braunwald and co-workers (240) coronary flow *in situ* is not only influenced by myocardial Q_{O_2} but also responds to changes in aortic pressure and cardiac output. Ventricular filling pressure did not affect either the Q_{O_2} or coronary flow. After administration of *l*-epinephrine and *l*-nor-epinephrine Feinberg & Katz (241) found coronary blood flow, myocardial oxygen availability, and coronary venous content to be consistently increased despite wide positive and negative fluctuations in heart rate and aortic pressure. Oxygen consumption still appears to be the primary determinant of coronary flow. Berglund and co-workers (242) confirm this statement. Coronary resistance fell as oxygen consumption increased; the coronary blood flow tended to parallel the heart rate. Verdun di Cantagno (243) reported that in innervated hearts greater work led to greater coronary flow and greater oxygen consumption. Vagal section abolished the compensation, a reflex which was considered vagovagal.

Kimura and co-workers have reverified the conclusion that coronary oxygen lack is the cause of electrocardiographic signs of injury (244) and find that the vagus acts as a coronary vasodilator even though arterial pressure and coronary flow are generally well correlated during vagal stimulation (245). Schreiner (246), however, found that when the heart rate was maintained constant, stimulating the vagus did not change myocardial flow. Mohme-Lundholm (247) presents evidence that epinephrine relaxes bovine coronary vessels through increased cellular lactic acid production. Marshak & Aronova (248) measured coronary flow in intact dogs thermoelectrically. Coronary flow increased on feeding, with administration of carbon dioxide, with rectal distention, with work, and at sight of a cat, and fell with pain.

Driscoll & Berne (249) found that, although elevating coronary arterial plasma potassium increased coronary blood flow, it is unlikely that potassium release is responsible for coronary flow changes accompanying altered cardiac metabolism. Reduced oxygen content of arterial blood did not influence coronary flow until the level fell below 5.5 volumes per cent. Vasodilation in hypoxemia is related to myocardial hypoxia according to Berne *et al.* (250). De Vera and co-workers (251) found that retrograde flow, which they equate with collateral, was less than 10 per cent of total coronary flow in 48 per cent of dogs studied. Laszt (252) presented results of extensive measurements of coronary flow. Of further importance are some anatomical studies previously cited [Paul *et al.* (16); Vastesaege *et al.* (15)]. Studies of coronary flow under a variety of experimental alterations of the circulation will be discussed later.

Jelliffe and co-workers (253), using a controlled perfusion technique, found no vasoactive substances in the coronary sinus blood of hypoxic "overperfused" hearts. Methods for catheterization of the coronary arteries in intact dogs were described by Horvath *et al.* (254) and West *et al.* (255). A new technique for possible cardiac revascularization developed by Day *et al.* (256) consists of submerging the bleeding end of a coronary artery in the myocardium.

HORMONAL EFFECTS

Collier (257) found that when infused into dogs, norepinephrine, unlike epinephrine and in contrast to effects of intravenous administration previously observed, decreased, or did not change cardiac output. Other effects were also contrary to the accepted idea that the dog and man respond differently to this agent. Lansing & Stevenson (258) report that norepinephrine counteracts hemorrhagic shock by affecting cardiac output rather than peripheral resistance. Effects of epinephrine, norepinephrine, and acetylcholine on the electrocardiogram of the perfused guinea pig heart were reported by Johansson & Vendsalu (259). Reidenberg *et al.* (260) found that adrenalectomy in dogs did not alter the ability to increase the force of cardiac contraction or arterial pressure in response to norepinephrine. Hollander and co-workers (261) described the cardiovascular effects of 5-hydroxytryptamine (serotonin). Holmes (262) has localized atrial cholinesterase to the muscle fibers, blood vessels, and nerve networks of the dog and cat.

According to Driscoll and co-workers (263) aldosterone secretion in dogs is not affected by experimental congestive heart failure.

Goh & Dallam (264) found that in normal animals, oxygen consumption per gram of tissue was greater in the left ventricle than in the right ventricle or either atrium. Animals with depressed thyroids had decreased oxygen consumption and those with increased thyroid activity had greater oxygen consumption. Selye (265) found that chlorocortisol treatment plus phosphate can produce focal myocardial lesions in rats. This effect is increased by certain fats (266). The steroid also conditions animals so that exertion can lead to similar lesions (267).

EXERCISE

Supine exercise in man produced no systematic alteration in the stroke index although increases in heart rate, arteriovenous oxygen difference, arterial pressure, and right atrial pressure were noted by Barratt-Boyes & Wood (268). It was felt that the relationship between venous oxygen saturation and cardiac output has a different setting during exercise. Kahn & Simonson (123) found differences between electrocardiographic changes in anaerobic exercise and in acute coronary insufficiency, and postulated a difference between generalized hypoxia and local ischemia. Exercised rats showed a marked increase in the ratio of heart weight to body weight, and in some species, differences between the sexes were noted by Van Liere *et al.* (269).

EXPERIMENTAL AND OTHER ALTERATIONS IN THE CIRCULATION

Braunwald & Morrow (270) reported no asynchrony between right and left ventricular contraction in many persons with bundle-branch block. This adds to the existing evidence that the electrocardiographic patterns ascribed to bundle-branch block in man are usually not the result of interruption of one of the main conducting bundles.

Roberson & Horvath (271) found that dogs subjected to unimpeded and unregulated bilateral carotid to jugular crossed circulation showed decreases in arterial pressure, changes in carotid shunt blood flow, heart rate, cardiac output, work, and other parameters although all animals recovered from the procedure. Ohara & Sakai (272) interrupted the thoracic inferior vena cava in normal and hypothermic dogs. If the obstruction was maintained 30 minutes, cardiac arrest followed release of the obstruction, and restoration of the circulation was followed by death in shock, possibly from hepatic pooling of blood. Hypothermia prevented the shock and death. Davis *et al.* (273) found that dogs with pulmonic stenosis developed increased right atrial pressure, sodium retention, and right heart failure if subjected to daily hemorrhage. Renal effects were also measured.

Braunwald and co-workers (274) found that mitral regurgitation of up to three times cardiac output had little effect on effective cardiac output, aortic or left atrial pressure, or on left ventricular function curves. Wegria and co-workers (275) found acute mitral insufficiency leads to greater coronary flow and greater oxygen consumption, caused either by decreased efficiency of the left ventricle or by the energy consumed in regurgitation. Moscovitz observed the pressure events of the cardiac cycle in dogs with mitral valvular lesions (276) and aortic lesions (277); aortic, left atrial, and left ventricular pressures were measured.

Wégria *et al.* (278) found lowered arterial pressure and an increase in coronary flow and myocardial oxygen consumption in experimental acute aortic insufficiency in dogs. Welch *et al.* (279) studied its hemodynamic effects experimentally. Regurgitation in excess of resting cardiac output caused a marked decrease in cardiac output, increase in peripheral resistance and end-diastolic pressure curves, and a depression of the ventricular function curve. Previous successful adaptation to moderately elevated aortic pressure

protected rabbits against congestive failure from severe aortic constriction according to Alexander & Drury (280). Unlike controls, protected animals could maintain the high arterial pressure needed for survival. Alexander *et al.* (281) found that aortic constriction sufficient to produce hypertensive failure leads to an increased plasma volume; with sufficient dehydration to prevent the plasma expansion, no increase in pressure is seen. The delay in onset of failure from this lesion they consider a result of the time required to increase plasma volume. Similar production of failure was reported by Franco (282).

Kuhn and co-workers (283) have studied the hemodynamics of human patent ductus arteriosus during surgical repair by recording pulmonary and aortic pressures. Węgria *et al.* (284) found that arteriovenous fistula increased coronary flow, work, oxygen consumption, and efficiency although arterial pressure fell. Mascitelli-Coriandoli and co-workers (285) subjected rats to high-intensity light and sound and found greatly increased heart weight and altered cardiac metabolism. Cardiac hypertrophy was noted by Sobel & Graboyes (286) in rats exposed to high temperature and altitude. Matejicek *et al.* (287) found hearts transplanted from one dog into another displayed abnormal electrical activity. A search for alterations in coronary venous nucleotides, amino acids, etc., in experimental occlusion was unsuccessful [Cabrera *et al.* (288)]. Szekeres *et al.* (289) found greater susceptibility to hypoxic failure of the left ventricle as compared to the right, and also greater oxygen consumption of left ventricular hemogenates. Simmons *et al.* (290) noted reflex effects of acute pneumothorax in dogs.

HYPOTHERMIA

Despite the fact that it no longer seems a useful adjunct to cardiac surgery, much research is being devoted to hypothermia. The procedure increases susceptibility to fibrillation and is associated with an increase in refractory period and a slower return to normal excitability although diastolic excitability is normal [Angelakos (291); Covino & Beavers (145)]. According to Covino & Beavers (146), previous acclimatization to cold has some protective effect, and Arnovljevic (292) finds differences between the responses of cooled animals and hibernating animals to further cooling, the latter showing smaller effects on depolarization and repolarization. Berne *et al.* (293) studied combinations of hypothermia with acetylcholine or potassium for possible use in elective surgical cardiac arrest. Potassium arrest followed by injection of cooled blood produced less fibrillation and more rapid recovery than other techniques. Angelakos *et al.* (294) reported on the susceptibility of the hypothermic myocardium to calcium-induced fibrillation. Electrocardiographic evidence of slower conduction has been reported by several workers including Datey *et al.* (295), Emslie-Smith (296), and Santos & Kittle (297). Badeer & Khachadurian (298) reported that the increased mechanical efficiency of the cooled heart performing constant work appears to be a direct temperature effect. A large series of detailed studies covers the general effects of hypothermia (299 to 305). Hunter (306) has defined conditions which aid in the reanimation of rats cooled to 2° to 3°C. for an hour. A surprising finding is the necessity for breathing gas high in carbon dioxide during cooling

if reanimation is to be successful. Hypothermia protects against shock from severe interference with the peripheral circulation [Ohara & Sakai (272)] or against death from previously lethal electric shock [Szilagyi *et al.* (307)]. Hannon & Covino (308) have studied the effects of hypothermia on the cellular respiration of ventricular slices and homogenates. General responses to hypothermia in man have been measured by Henneman *et al.* (309).

EFFECTS OF DRUGS

Gilbert *et al.* (310) found that a large number of vasoconstrictors induced ectopic beats; methoxamine, on the other hand, increased the duration of the action potential and the refractory period, and slowed conduction velocity. Cotten and co-workers (311) tested adrenergic blocking agents as inhibitors of the increased cardiac contractile force produced by sympathomimetic amines in dogs. Isolated atria inhibited by anticholinesterases can be reactivated with atropine and epinephrine according to Katsh (312). The potentiating effects of iproniazid on the depressor response of histamine were studied by Lindell (313). Oppenheimer and co-workers (314) found that mephentermine increases conduction velocity and shortens refractory period, properties which they feel may make it clinically useful. Nahas (315) observed that hydroxycortisone, if given 12 to 15 min. before carbon dioxide, mitigates the acidotic failure usually seen in the denervated heart-lung preparation. Benforado (316) found that some cardioactive drugs affected resting and active tension reciprocally in the rat ventricle. Effects of these are dependent on frequency of stimulation among other parameters.

Bennet and co-workers (317) investigated the structure and function of simple digitalislike compounds. Mendez & Mendez (85) found that cardiac glycosides induced a variety of effects on excitability and velocity which were greater in atrial tissues and were in part not dependent on the vagus. A relationship between the frequency of stimulation and rate of effect of ouabain demonstrated by Sanyal & Saunders (318) led them to conclude that ouabain can affect cardiac muscle only when the muscle is in the active state. Ouabain improved ventricular function curves, increased contractile force, peripheral resistance and arterial pressure, and decreased cardiac output rate and atrial pressures [Cotten & Stopp (319)]. Caviezel & Wilbrandt (320) reported evidence that cardiac glycosides compete metabolically for some potassium steroid. Smith (321) found that ouabain increased the oxygen consumption of chick heart when alterations of potassium and sodium were also induced. Ouabain and veratridine effects on potassium movement in the perfused guinea pig heart were studied by Vick & Kahn (322). Gaffney and co-workers (323) have confirmed earlier findings that ouabain sensitizes the heart to vagal stimulation and acetylcholine. Berman (324) has reported that glucose was much more effective in sustaining the contractile activity of the myocardium when ouabain was also given. Ouabain apparently increases the conversion of glucose to pyruvate and facilitates energy production by the heart. Rebar and co-workers (325) found that digitoxin in nontoxic doses lowers creatine phosphate and raises lactate in dog left ventricle: glyco-

gen was also altered. Effects on potassium content depended on dosage.

Hess & Haugaard (326) found that quinidine and quinine inhibit the oxygen utilization of rat heart *in vitro* but that chloroquine and procaine do not have this effect. Johnson & Robertson (327) explain the atrial action of quinidine as probably resulting from sodium carrier inactivation. Williams (328) has interpreted the action of quinidine from studies with intracellular electrodes. Holland (329), noting that the effects of quinidine on rabbit atria can be enhanced by elevating potassium, has suggested that quinidine depresses outward potassium flux and finds that transport studies are in accord. He further analyzes the hypothesis that quinidine blocks intracellularly released acetylcholine. Robertson *et al.* (330) find that pentobarbital has a selective effect on control of intracellular sodium and water in cat atrium but not in cat or dog ventricle. Blatteis & Horvath (331) reported that arterial pressure was not affected by pentobarbital anesthesia although heart rate increased significantly. This study was primarily directed toward elucidating the renal effects of the anesthetic. Laforet *et al.* (84) found significant although slight increases in refractoriness with pentobarbital in dogs. Karczmar and co-workers (332) studied the properties of the polymethoxyphenyl drugs which are, among other effects, antiacceleratory and vasodilator. Swain & Weidner (333) and Swain & McCarthy (334) studied a number of substances, including veratrine alkaloids, andromedotoxin, and cardiac glycosides, which decrease conduction velocity in Purkinje fibers while myocardial velocity is unaltered or increased. Electrocardiographic alterations induced by stingray venom were reported by Russell *et al.* (335).

LITERATURE CITED

1. Huxley, H. E., *J. Biophys. Biochem. Cytol.*, **3**, 631-47 (1957)
2. Muir, A. R., *J. Biophys. Biochem. Cytol.*, **3**, 193-201 (1957)
3. Sjöstrand, F. S., Andersson-Cedergren, E., and Dewey, M. M., *J. Ultrastructure Research*, **1**, 271-87 (1958)
4. Moore, D. H., and Ruska, H., *J. Biophys. Biochem. Cytol.*, **3**, 261-67 (1957)
5. Fawcett, D. W., and Selby, C. C., *J. Biophys. Biochem. Cytol.*, **4**, 23-29, 63-72, (1958)
6. Schoenmackers, J., *Arch. pathol. Anat. u. Physiol., Virchows'*, **331**, 3-22 (1958)
7. Oken, D. E., and Boucek, R. J., *Circulation Research*, **5**, 357-61 (1957)
8. Thomas, C. E., *Am. J. Anat.*, **101**, 17-57 (1957)
9. Northup, D. W., Van Lier, E. J., and Stickney, J. C., *Anat. Record*, **128**, 411-18 (1957)
10. Keen, E. N., and Goetz, R. H., *Acta Anat.*, **31**, 562-71 (1957)
11. Sharma, H. L., *J. Morphol.*, **100**, 313-43 (1957)
12. James, T. N., and Burch, G. E., *Circulation*, **17**, 391-96 (1958)
13. James, T. N., and Burch, G. E., *Circulation*, **17**, 90-98 (1958)
14. Halpern, M. H., *Am. J. Anat.*, **101**, 1-16 (1957)
15. Vastesaegeer, M. M., Van Der Straeten, P. P., Friart, J., Candaele, G., Ghys, A., and Bernard, R. M., *Acta cardiol.*, **12**, 365-401 (1957)
16. Paul, M. H., Norman, L. R., Zoll, P. M., and Blumgart, H. L., *Circulation*, **16**, 608-14 (1957)
17. Prakash, R., *Proc. Natl. Inst. Sci. India*, **22**, 255-58 (1956)

18. Prakash, R., *Sci. and Culture (Calcutta)*, **22**, 567-58 (1957)
19. Bhatnagar, S. P., *Indian J. Med. Sci.*, **11**, 410-14 (1957)
20. Köberle, F., *Arch. pathol. Anat. u. Physiol., Virchow's*, **330**, 455-58 (1958)
21. Gresham, G. A., *Brit. Heart J.*, **19**, 381-86 (1957)
22. Coleridge, J. C. G., Hemingway, A., Holmes, R. L., and Linden, R. J., *J. Physiol. (London)*, **136**, 174-97 (1957)
23. Kolatat, T., Kramer, K., and Mühl, N., *Arch. ges. Physiol.*, **264**, 127-44 (1957)
24. White, J. C., *Circulation*, **16**, 644-55 (1957)
25. Waites, G. M. H., *J. Physiol. (London)*, **139**, 417-33 (1957)
26. Kato, G., Ito, S., and Omi, I., *Japan. J. Physiol.*, **8**, 67-75 (1958)
27. Kato, G., Ito, S., and Sakakibara, R., *Japan. J. Physiol.*, **8**, 76-82 (1958)
28. Lorber, V., *Ann. Rev. Physiol.*, **20**, 97-122 (1957)
29. Duffey, L. M., and Ebert, J. D., *J. Embryol. and Exptl. Morphol.*, **5**, 324-39 (1958)
30. Lukens, F. D. W., *Am. J. Physiol.*, **192**, 485-90 (1958)
31. Fawaz, G., Tutunji, B., and Fawaz, E. N., *Proc. Soc. Exptl. Biol. Med.*, **97**, 770-73 (1958)
32. Hollander, P. B., and Webb, J. L., *Circulation Research*, **5**, 349-53 (1957)
33. Fleckenstein, A., Hochrein, H., and Kotowski, H., *Arch. ges. Physiol.*, **265**, 485-587 (1958)
34. Macfarlane, W. V., *Australasian Ann. Med.*, **6**, 269-76 (1957)
35. Grauer, H., *Cardiologia*, **31**, 86-109 (1957)
36. Angelucci, L., Boldrini, R., and Mascitelli-Coriandoli, E., *Nature*, **181**, 419-20 (1958)
37. Burdette, W. J., *Am. Heart J.*, **54**, 746-52 (1957)
38. Raiskina, M. E., *Bull. Exptl. Biol. Med.*, **41**, 421-24 (1956)
39. Hess, M. E., and Haugaard, N., *J. Pharmacol. Exptl. Therap.*, **122**, 169-75 (1958)
40. Hearn, G. R., and Wainio, W. W., *Am. J. Physiol.*, **190**, 206-8 (1957)
41. Penna, M., Illanes, A., Rivera, J., and Mardones, J., *Acta Physiol. Latinoam.*, **7**, 110-16 (1957)
42. Jordan, P., Jr., Tolstedt, G. E., and Beretta, F. F., *Surg., Gynecol. Obstet.*, **105**, 615-20 (1957)
43. Picard, D., Chambost, G., and Vitry, G., *Acta Anat.*, **30**, 613-23 (1957)
44. Van Citters, R. L., Ruth, W. E., and Reissmann, K. R., *Am. J. Physiol.*, **191**, 443-45 (1957)
45. Sarnoff, S. J., Case, R. B., Welch, G. H., Jr., Braunwald, E., and Stainsby, W. N., *Am. J. Physiol.*, **192**, 141-47 (1958)
46. Sarnoff, S. J., Braunwald, E., Welch, G. H., Jr., Case, R. B., Stainsby, W. N., and Macruz, R., *Am. J. Physiol.*, **192**, 148-56 (1958)
47. Bretschneider, H. J., Frank, A., Kansow, E., and Bernard, U., *Arch. ges. Physiol.*, **264**, 399-423 (1957)
48. Berglund, E., Monroe, R. G., and Schreiner, G. L., *Acta Physiol. Scand.*, **41**, 261-68 (1957)
49. Beuren, A., Sparks, C., and Bing, R. J., *Am. J. Cardiol.*, **1**, 103-12 (1958)
50. Schreiner, G. L., Berglund, E., Borst, H. G., and Monroe, R. G., *Circulation Research*, **5**, 562-67 (1957)
51. Whalen, W. J., *Circulation Research*, **5**, 556-61 (1957)
52. Paff, G. H., and Boucek, R. J., *Circulation Research*, **6**, 88-91 (1958)
53. Nahas, G. G., and Cavert, H. M., *Am. J. Physiol.*, **190**, 483-91 (1957)
54. Niedergerke, R., *J. Physiol. (London)*, **138**, 506-15 (1957)

55. Merrick, A. W., *Proc. Soc. Exptl. Biol. Med.*, **96**, 592-96 (1957)
56. Johnson, J. A., *Am. J. Physiol.*, **191**, 487-92 (1957)
57. Caviezel, R., Koller, H., and Wilbrandt, W., *Helv. Physiol. et Pharmacol. Acta*, **16**, 22-30 (1958)
58. Beznák, A. B. L., *Nature*, **181**, 1190-92 (1958)
59. Cranefield, P. F., and Hoffman, B. F., *Physiol. Revs.*, **38**, 41-76 (1958)
60. Hecht, H. H., Ed., *Ann. N. Y. Acad. Sci.*, **65**, 653-1146 (1957)
61. Weidmann, S., *Elektrophysiologie der Herzmuskelfaser* (Japanese trans., Matsuda, K., Kinpo-do, Kyoto, Tokyo, Japan, 106 pp., 1957)
62. Weidmann, S., *Cardiologia*, **31**, 186-94 (1957)
63. Shanes, A. M., *Pharmacol. Revs.*, **10**, 59-164 (1958)
64. Murphy, Q. R., Ed., *Metabolic Aspects of Transport Across Cell Membranes* (University of Wisconsin Press, Madison, Wis., 379 pp., 1957)
65. Weidmann, S., *Ann. N. Y. Acad. Sci.*, **65**, 663-78 (1957)
66. Brady, A. J., and Woodbury, J. W., *Ann. N. Y. Acad. Sci.*, **65**, 687-92 (1957)
67. Hecht, H. H., *Ann. N. Y. Acad. Sci.*, **65**, 700-33 (1957)
68. Wilde, W. S., *Ann. N. Y. Acad. Sci.*, **65**, 693-99 (1957)
69. Coraboeuf, E., Distel, R., and Boistel, J., *Microphysiol. comparée des éléments excitables*, **67**, 123-45 (1955)
70. Ware, F., Jr., Bennett, A. L., and McIntyre, A. R., *Am. J. Physiol.*, **190**, 194-200 (1957)
71. Gargoull, Y., and Coraboeuf, E., *Compt. rend.*, **245**, 1949-52 (1957)
72. Hoffman, B. F., Paes de Carvalho, A., and Carlos de Mella, W., *Nature*, **181**, 66-67 (1958)
73. Woodbury, J. W., Lee, J., Brady, A. J., and Merendino, K. A., *Circulation Research*, **5**, 179 (1957)
74. Carmeliet, E., and Lacquet, L., *Arch. intern. physiol. et biochem.*, **66**, 1-21 (1958)
75. Trautwein, W., and Dudel, J., *Arch. ges. Physiol.*, **266**, 324-34 (1958)
76. Marshall, J. M., *Circulation Research*, **6**, 664-69 (1957)
77. Tasaki, I., and Hagiwara, S., *J. Gen. Physiol.*, **41**, 859-85 (1957)
78. Otsuka, M., *Arch. ges. Physiol.*, **266**, 512-17 (1958)
79. Otsuka, M., and Gürtner, H. P., *Helv. Physiol. et Pharmacol. Acta*, **15**, 73-75 (1957)
80. Cranefield, P. F., and Hoffman, B. F., *J. Gen. Physiol.*, **41**, 633-49 (1958)
81. Cranefield, P. F., Hoffman, B. F., and Siebens, A. A., *Am. J. Physiol.*, **190**, 383-90 (1957)
82. Hoffman, B. F., Kao, C. Y., and Suckling, E. E., *Am. J. Physiol.*, **190**, 473-82 (1957)
83. Ullrick, W. C., and Sommers, S. C., *Lab. Invest.*, **6**, 528-35 (1957)
84. Laforet, E. G., Angelakos, E. T., and Hegnauer, A. H., *Am. J. Physiol.*, **189**, 596-98 (1957)
85. Mendez, C., and Mendez, R., *J. Pharmacol. Exptl. Therap.*, **121**, 402-13 (1957)
86. Scher, A. M., Liikane, J., Rodriguez, M. I., and Young, A. C., *Science*, **127**, 873-74 (1958)
87. Deutsch, E., *Arch. inst. cardiol. Mex.*, **28**, 97-117 (1958)
88. Burchell, H. B., *Ann. N. Y. Acad. Sci.*, **65**, 741-42 (1957)
89. Scher, A. M., and Young, A. C., *Ann. N. Y. Acad. Sci.*, **65**, 768-78 (1957)
90. Durrer, D., and van der Tweel, L. H., *Ann. N. Y. Acad. Sci.*, **65**, 779-803 (1957)
91. Medrano, G. A., Bisteni, A., Brancato, R. W., Pileggi, F., and Sodi-Pallares, D., *Ann. N. Y. Acad. Sci.*, **65**, 804-17 (1957)

92. Medrano, G. A., Pileggi, F., Sotomayor, A., Bisteni, A., and Sodi-Pallares, D., *Arch. inst. cardiol. Mex.*, **26**, 616-43 (1956)
93. Medrano, G. A., Pileggi, F., Sotomayor, A., Bisteni, A., and Sodi-Pallares, D., *Arch. inst. cardiol. Mex.*, **27**, 299-322 (1957)
94. Medrano, G. A., Pileggi, F., Sotomayor, A., Bisteni, A., and Sodi-Pallares, D., *Arch. inst. cardiol. Mex.*, **27**, 609-44 (1957)
95. Erickson, R. V., Scher, A. M., and Becker, R. A., *Circulation Research*, **5**, 5-10 (1957)
96. Becker, R. A., Scher, A. M., and Erickson, R. V., *Am. Heart J.*, **55**, 547-56 (1958)
97. Frimpter, G. W., Scherr, L., and Ogden, D., *Am. Heart J.*, **55**, 220-30 (1958)
98. Dubouloz, P., *Cardiologia*, **32**, 193-211 (1958)
99. Pipberger, H., Schwartz, L., Massumi, R. A., Weiner, S. M., and Prinzmetal, M., *Am. Heart J.*, **54**, 511-30 (1957)
100. Johnston, F. D., *Circulation*, **15**, 801-4 (1957)
101. Sodi-Pallares, D., Brancato, R. W., Pileggi, F., Medrano, G. A., Bisteni, A., and Barbato, E., *Am. Heart J.*, **54**, 498-510 (1957)
102. Pipberger, H., Schwartz, L., Massumi, R. A., and Prinzmetal, M., *Ann. N. Y. Acad. Sci.*, **65**, 924-31 (1957)
103. Ferrero, C., *Cardiologia*, **31**, 296-301 (1957)
104. Burger, H. C., *Ann. N. Y. Acad. Sci.*, **65**, 1076-87 (1957)
105. Okada, R. H., *Science*, **127**, 240-41 (1958)
106. Katz, L. N., *Ann. N. Y. Acad. Sci.*, **65**, 960-62 (1957)
107. Schmitt, O. H., *Ann. N. Y. Acad. Sci.*, **65**, 1092-1109 (1957)
108. Frank, E., *Ann. N. Y. Acad. Sci.*, **65**, 980-1002 (1957)
109. Burch, G. E., *Ann. N. Y. Acad. Sci.*, **65**, 1073-75 (1957)
110. Wolferth, C. C., *Circulation*, **16**, 321-22 (1957)
111. Okada, R. H., *Am. Heart J.*, **54**, 567-71 (1957)
112. Reynolds, E. W., Jr., Cordes, J. F., Willis, P. W., III, and Johnston, F. D., *Circulation*, **14**, 48-54 (1956)
113. Abildskov, J. A., *Ann. N. Y. Acad. Sci.*, **65**, 903-4 (1957)
114. Pipberger, H. V., and Wood, C. R., Jr., *Circulation Research*, **6**, 239-43 (1958)
115. Helm, R. A., *Am. Heart J.*, **54**, 89-97 (1957)
116. Helm, R. A., *Am. Heart J.*, **54**, 602-4 (1957)
117. Whipple, G. H., *Med. Clin. N. Am.*, **41**, 1193-1214 (1957)
118. Milnor, W. R., *Circulation*, **16**, 95-106 (1957)
119. Langner, P. H., Jr., Okada, R. H., Moore, S. R., and Fies, H. L., *Circulation*, **17**, 46-54 (1958)
120. Seiden, G. E., *Circulation*, **16**, 582-85 (1957)
121. Duchosal, P. W., and Moret, P., *Cardiologia*, **32**, 129-54 (1958)
122. Simonson, E., *Am. Heart J.*, **55**, 80-103 (1958)
123. Kahn, K. A., and Simonson, E., *Circulation Research*, **5**, 629-33 (1957)
124. Lamb, L. E., *Am. Heart J.*, **54**, 342-51 (1957)
125. Simonson, E., Nakagawa, K., and Schmitt, O. H., *Am. Heart J.*, **54**, 919-39 (1957)
126. Brinberg, L., *Am. J. Med.*, **23**, 212-25 (1957)
127. Gärtner, W., and Schaefer, H., *Arch. Krieslaufforsch.*, **27**, 83-117 (1957)
128. Gardberg, M., and Rosen, I. L., *Ann. N. Y. Acad. Sci.*, **65**, 873-93 (1957)
129. Briller, S. A., *Ann. N. Y. Acad. Sci.*, **65**, 894-902 (1957)
130. Brody, D. A., *Circulation Research*, **5**, 522-26 (1957)

131. Horan, L., Burch, G. E., and Cronvich, J. A., *Circulation Research*, **5**, 133-36 (1957)
132. Irisawa, H., and Irisawa, A. F., *Biol. Bull.*, **112**, 358-62 (1957)
133. Schwan, H. P., and Kay, C. F., *Ann. N. Y. Acad. Sci.*, **65**, 1007-13 (1957)
134. Schwan, H. P., and Kay, C. F., *Circulation Research*, **5**, 439-43 (1957)
135. Bayley, R. H., *Ann. N. Y. Acad. Sci.*, **65**, 1110-34 (1957)
136. Donoso, E., Wachtel, R., and Grishman, A., *Am. J. Physiol.*, **189**, 214-23 (1957)
137. Alzamora-Castro, V., Battilana, G., and Abugattas, R., *Am. Heart J.*, **54**, 254-63 (1957)
138. Kennamer, R., Miles, E., and Prinzmetal, M., *Clin. Sci.*, **16**, 451-61 (1957)
139. Kimura, E., Kato, K., Murao, S., Ajisaka, H., Koyama, S., and Omiya, Z., *Tôhoku J. Exptl. Med.*, **50**, 197-207 (1954)
140. Kato, K., Sato, M., Harumi, K., Murao, S., Kanazawa, T., Hanzawa, S., and Kimura, E., *Tôhoku J. Exptl. Med.*, **64**, 377-85 (1956)
141. Scherf, D., Blumenfeld, S., Chamsai, D. G., Reid, E. C., and Gürbüz, B., *Am. Heart J.*, **54**, 561-66 (1957)
142. Scherf, D., Blumenfeld, S., Gürbüz, B., and Jody, A., *Am. Heart J.*, **55**, 733-38 (1958)
143. Angelakos, E. T., and Shepherd, G. M., *Circulation Research*, **5**, 657-58 (1957)
144. Sano, T., Tsuchihashi, H., and Shimamoto, T., *Circulation Research*, **6**, 41-46 (1958)
145. Covino, B. G., and Beavers, W. R., *Proc. Soc. Exptl. Biol. Med.*, **95**, 631-34 (1957)
146. Covino, B. G., and Beavers, W. R., *Am. J. Physiol.*, **191**, 153-56 (1957)
147. Milnor, W. R., Knickerbocker, G. G., and Kouwenhoven, W. V., *Circulation Research*, **6**, 60-65 (1958)
148. Maling, H. M., and Moran, N. C., *Circulation Research*, **5**, 409-13 (1957)
149. Kärki, N. T., *J. Physiol. (London)*, **141**, 366-76 (1958)
150. Steffenson, J. L., and Murphy, Q. R., *Proc. Soc. Exptl. Biol. Med.*, **98**, 155-57 (1958)
151. Cherbakoff, A., Toyama, S., and Hamilton, W. F., *Circulation Research*, **5**, 517-21 (1957)
152. Carden, N. L., and Steinhaus, J. E., *Circulation Research*, **5**, 405-8 (1957)
153. Grumbach, L., *Circulation Research*, **5**, 362-67 (1957)
154. Klien, R. L., and Holland, W. C., *Am. J. Physiol.*, **193**, 239-43 (1958)
155. Holland, W. C., and Tinsley, B., *Am. J. Physiol.*, **193**, 235-38 (1958)
156. Brown, E. B., Jr., and Prasad, A. S., *Am. J. Physiol.*, **190**, 462-66 (1957)
157. Ringer, R. K., Weiss, H. S., and Sturkie, P. D., *Am. J. Physiol.*, **191**, 145-47 (1957)
158. Pathak, C. L., *Am. J. Physiol.*, **192**, 111-13 (1958)
159. Mainwood, G. W., *Can. J. Biochem. and Physiol.*, **35**, 1153-64 (1957)
160. Wilhelmj, C. M., Carnazzo, A. J., and McCarthy, H. H., *Am. J. Physiol.*, **191**, 103-7 (1957)
161. Penaz, J., *Arch. intern. physiol. et biochem.*, **65**, 306-14 (1957)
162. Lester, D., *J. Appl. Physiol.*, **11**, 489-90 (1957)
163. Hajdu, S., Weiss, H., and Titus, E., *J. Pharmacol. Exptl. Therap.*, **120**, 99-113 (1957)
164. Green, J. P., and Nahum, L. H., *Circulation Research*, **6**, 634-40 (1957)
165. Cobbin, L. B., and Thorp, R. H., *Nature*, **180**, 242-43 (1957)
166. Cornman, I., and Gargus, J. L., *Am. J. Physiol.*, **189**, 347-49 (1957)

167. Cornman, I., MacDonald, M., and Trams, E., *Am. J. Physiol.*, **189**, 350-54 (1957)
168. Benson, E. S., Hallaway, B. E., and Turbak, C. E., *Circulation Research*, **6**, 122-28 (1958)
169. Kako, K., and Bing, R. J., *J. Clin. Invest.*, **37**, 465-70 (1958)
170. Whalen, W. J., *Science*, **127**, 468-69 (1958)
171. Gersch, M., and Deuse, R., *Arch. ges. Physiol.*, **265**, 11-17 (1957)
172. Bucher, O., *Acta Anat.*, **30**, 158-66 (1957)
173. Brandt, W., *Acta Anat.*, **30**, 128-32 (1957)
174. Burch, G. E., Cronvich, J. A., Creech, O., and Hyman, A., *Am. Heart J.*, **53**, 890-94 (1957)
175. Fowler, N. O., Bloom, W. L., and Ferris, E. B., *Circulation Research*, **5**, 485-88 (1957)
176. Brecher, G. A., and Kissen, A. T., *Circulation Research*, **5**, 157-62 (1957)
177. Brecher, G. A., and Kissen, A. T., *Circulation Research*, **6**, 100-6 (1958)
178. Braunwald, E., Sarnoff, S. J., and Stainsby, W. N., *Circulation Research*, **6**, 319-25 (1958)
179. Cotten, M. de V., and Maling, H. M., *Am. J. Physiol.*, **189**, 580-86 (1957)
180. Mazzella, H., *Acta Physiol. Latinoam.*, **7**, 104-9 (1957)
181. Guyton, A. C., Lindsey, A. W., Abernathy, B., and Loangston, J. B., *Am. J. Physiol.*, **192**, 126-29 (1958)
182. Roy, S. B., Gadboys, H. L., and Dow, J. W., *Am. Heart J.*, **54**, 753-65 (1957)
183. Oppenheimer, M. J., and Stauffer, H. M., *J. Appl. Physiol.*, **12**, 324-28 (1958)
184. Paul, R. E., Oppenheimer, M. J., Lynch, P. R., and Stauffer, H. M., *J. Appl. Physiol.*, **12**, 98-104 (1958)
185. Attinger, E. O., *Am. Heart J.*, **54**, 837-50 (1957)
186. Dubois, A. B., and Marshall, R., *J. Clin. Invest.*, **36**, 1566-71 (1957)
187. Figley, M. M., *Am. J. Med. Sci.*, **234**, 613-30 (1957)
188. McKusick, V. A., *Circulation*, **16**, 270-90 (1957)
189. Siecke, H., and Essex, H. E., *Am. J. Physiol.*, **191**, 469-75 (1957)
190. Siecke, H., and Essex, H. E., *Am. J. Physiol.*, **192**, 135-40 (1958)
191. Reinhold, J., and Rudhe, U., *Brit. Heart J.*, **19**, 473-85 (1957)
192. Lewis, D. H., Deitz, G. W., Wallace, J. D., and Brown, J. R., Jr., *Circulation*, **16**, 764-75 (1957)
193. Groom, D., and Sihvonen, Y. T., *Am. Heart J.*, **54**, 592-601 (1957)
194. Luisada, A. A., and Liu, C. K., *Am. Heart J.*, **54**, 531-36 (1957)
195. Grant, R. P., *Circulation*, **16**, 414-36 (1957)
196. Mannheimer, E., *Am. Heart J.*, **54**, 314-15 (1957)
197. Wood, E. H., Swan, H. J. C., and Helmholtz, H. F., Jr., *Proc. Staff Meetings Mayo Clinic*, **32**, 464-77 (1957)
198. Wood, E. H., and Woodward, E., Jr., *Proc. Staff Meetings Mayo Clinic*, **32**, 536-41 (1957)
199. Wright, J. L., and Wood, E. H., *Proc. Staff Meetings Mayo Clinic*, **32**, 491-95 (1957)
200. Fox, I. J., and Wood, E. H., *Proc. Staff Meetings Mayo Clinics*, **32**, 541-50 (1957)
201. Fox, I. J., Brooker, L. G. S., Heseltine, D. W., Essex, H. E., and Wood, E. H., *Proc. Staff Meetings Mayo Clinic*, **32**, 478-84 (1957)
202. Fox, I. J., Sutterer, W. F., and Wood, E. H., *J. Appl. Physiol.*, **11**, 390-404 (1957)
203. Lacy, W. W., Emanuel, R. W., and Newman, E. V., *Circulation Research*, **5**, 568-72 (1957)

204. Emanuel, R. W., Lacy, W. W., and Newman, E. V., *Circulation Research*, **5**, 527-30 (1957)
205. Grace, J. B., Fox, I. J., Crowley, W. P., Jr., and Wood, E. H., *J. Appl. Physiol.*, **11**, 405-18 (1957)
206. Fitts, H. W., Jr., Harris, P., Chidsey, C. A., III, Clauss, R. H., and Cournand, A., *J. Appl. Physiol.*, **11**, 362-64 (1957)
207. Keys, J. R., Hetzel, P. S., and Wood, E. H., *J. Appl. Physiol.*, **11**, 385-89 (1957)
208. Goodwin, R. S., and Sapirstein, L. A., *Circulation Research*, **5**, 531-38 (1957)
209. Lochner, W., and dal Ri, H., *Arch. ges. Physiol.*, **264**, 543-48 (1957)
210. Booth, R. W., Ryan, J. M., and Goodwin, R. S., *Circulation Research*, **6**, 142-45 (1957)
211. Conn, H. L., Jr., Joyner, C. R., Heiman, D. F., and Zinsser, H. F., *Circulation Research*, **5**, 498-503 (1957)
212. Hayden, D. T., Garrett, W., and Jordan, P., *Circulation Research*, **6**, 77-82 (1958)
213. Warner, H. R., and Toronto, A. F., *Circulation Research*, **6**, 29-34 (1958)
214. Huff, R. L., Parrish, D., and Crockett, W., *Circulation Research*, **5**, 395-400 (1957)
215. Scarborough, W. R., and Baker, B. M., *Circulation*, **16**, 971-75 (1957)
216. Klensch, H., and Hohnen, H. W., *Arch. ges. Physiol.*, **265**, 207-19 (1957)
217. Klensch, H., Hohnen, H. W., and Kesseler, K. H., *Arch. ges. Physiol.*, **264**, 424-40 (1957)
218. Hohnen, H. W., and Klensch, H., *Arch. ges. Physiol.*, **265**, 199-206 (1957)
219. Scarborough, W. R., *Am. Heart J.*, **54**, 651-77 (1957)
220. Reeves, T. J., Jones, W. B., and Hefner, L. L., *Circulation*, **16**, 36-42 (1957)
221. Frederick, W. H., and Eddleman, E. E., Jr., *J. Appl. Physiol.*, **12**, 347-48 (1958)
222. Reeves, T. J., Hefner, L. L., Jones, W. B., and Sparks, J. E., *Circulation*, **16**, 43-53 (1957)
223. Starr, I., and Schild, A., *J. Appl. Physiol.*, **11**, 169-73 (1957)
224. Porje, I. G., and Rudewald, B., *Cardiologia*, **31**, 389-400 (1958)
225. Evans, R. L., *Nature*, **181**, 1471 (1958)
226. Brotmacher, L., *Circulation Research*, **5**, 589-93 (1957)
227. Lüthy, E., Gürtler, R., Hünemeyer, F., and Schmid, F., *Cardiologia*, **31**, 253-57 (1957)
228. Starr, I., *Circulation*, **17**, 589-600 (1958)
229. Barer, G. R., and Nüsser, E., *J. Physiol. (London)*, **138**, 103-18 (1957)
230. Barratt-Boyes, B. G., and Wood, E. H., *J. Lab. Clin. Med.*, **50**, 93-106 (1957)
231. Weissler, A. M., Leonard, J. J., and Warren, J. V., *J. Clin. Invest.*, **36**, 1656-62 (1957)
232. Weil, P., Salisbury, P. F., and State, D., *Am. J. Physiol.*, **191**, 453-60 (1957)
233. Rushmer, R. F., *Am. J. Physiol.*, **192**, 631-34 (1958)
234. Randall, W. C., McNally, H., Cowan, J., Califuri, L., and Rohse, W. G., *Am. J. Physiol.*, **191**, 213-17 (1957)
235. Cotten, M. de V., and Moran, N. C., *Am. J. Physiol.*, **191**, 461-68 (1957)
236. Lovegrove, T. D., Gowdey, C. W., and Stevenson, A. F., *Circulation Research*, **5**, 659-63 (1957)
237. Rohse, W. G., Kaye, M., and Randall, W. C., *Circulation Research*, **5**, 144-48 (1957)
238. Carlsten, A., Falkow, B., and Hamberger, C. A., *Acta Physiol. Scand.*, **41**, 68-76 (1957)
239. Koepchen, H. P., and Thureau, K., *Arch. ges. Physiol.*, **264**, 573-84 (1957)

240. Braunwald, E., Sarnoff, S. J., Case, R. B., Stainsby, W. N., and Welch, G. H., Jr., *Am. J. Physiol.*, **192**, 157-63 (1958)
241. Feinberg, H., and Katz, L. N., *Am. J. Physiol.*, **193**, 151-56 (1958)
242. Berglund, E., Borst, H. G., Duff, F., and Schreiner, G. L., *Acta Physiol. Scand.*, **42**, 185-98 (1958)
243. Verdun di Cantogno, L., and Ramirez de Arellano, J., *Arch. inst. cardiol. Mex.*, **28**, 81-96 (1958)
244. Kimura, E., Suzuki, N., Kanazawa, T., Ito, Y., Harigai, N., Yamamoto, F., Kumagai, S., Suzuki, Y., and Obara, F., *Tôhoku J. Exptl. Med.*, **66**, 25-32 (1957)
245. Kimura, E., Kanazawa, T., Suzuki, N., Ito, Y., Harigai, N., and Yamamoto, F., *Tôhoku J. Exptl. Med.*, **66**, 33-41 (1957)
246. Schreiner, G. L., Berglund, E., Borst, H. G., and Monroe, R. G., *Circulation Research*, **5**, 562-67 (1957)
247. Mohme-Lundholm, E., *Acta Physiol. Scand.*, **38**, 255-64 (1957)
248. Marshak, M. E., and Aronova, G. N., *Bull. Exptl. Biol. Med.*, **1**, 1-3 (1957)
249. Driscoll, T. E., and Berne, R. M., *Proc. Soc. Exptl. Biol. Med.*, **96**, 505-8 (1957)
250. Berne, R. M., Blackmon, J. R., and Gardner, T. H., *J. Clin. Invest.*, **36**, 1101-6 (1957)
251. De Vera, L. B., Gold, H., and Corday, E., *Circulation Research*, **6**, 26-28 (1958)
252. Laszt, L., and Müller, A., *Helv. Physiol. et Pharmacol. Acta*, **15**, 38-54 (1957)
253. Jelliffe, R. W., Wolf, C. R., Berne, R. M., and Eckstein, R. W., *Circulation Research*, **5**, 382-87 (1957)
254. Horvath, S. M., Ferrand, E. A., Blatteis, C., and Everingham, A., *Am. Heart J.*, **54**, 138-45 (1957)
255. West, J. W., Kobayashi, T., and Guzman, S. V., *Circulation Research*, **6**, 383-88 (1958)
256. Day, S. B., Yonehiro, E. G., Root, H. D., Crisp, N. W., Jr., Jenson, C., and Wangenstein, O. H., *Proc. Soc. Exptl. Biol. Med.*, **97**, 877-79 (1958)
257. Collier, H. D., Meyers, F. H., and Schmitt, G. H., *Am. J. Physiol.*, **189**, 224-28 (1957)
258. Lansing, A. M., and Stevenson, J. A. F., *Am. J. Physiol.*, **193**, 289-93 (1958)
259. Johansson, B., and Vendsalu, A., *Acta Physiol. Scand.*, **39**, 356-69 (1957)
260. Reidenberg, M. M., Ohler, E. A., and Sevy, R. W., *Proc. Soc. Exptl. Biol. Med.*, **97**, 889-92 (1958)
261. Hollander, W., Michelson, A. L., and Wilkins, R. W., *Circulation*, **16**, 246-55 (1957)
262. Holmes, R. L., *J. Physiol. (London)*, **137**, 421-26 (1957)
263. Driscoll, T. E., Maultsby, M. M., Farrell, G. L., and Berne, R. M., *Am. J. Physiol.*, **191**, 140-44 (1957)
264. Goh, K., and Dallam, R. D., *Am. J. Physiol.*, **188**, 514-18 (1957)
265. Selye, H., and Renaud, S., *Am. J. Cardiol.*, **1**, 208-11 (1958)
266. Selye, H., *Proc. Soc. Exptl. Biol. Med.*, **98**, 61-62 (1958)
267. Selye, H., and Renaud, S., *Proc. Soc. Exptl. Biol. Med.*, **96**, 512-14 (1957)
268. Barratt-Boyes, B. G., and Wood, E. H., *J. Appl. Physiol.*, **11**, 129-35 (1957)
269. Van Lier, E. J., and Northup, D. W., *J. Appl. Physiol.*, **11**, 91-92 (1957)
270. Braunwald, E., and Morrow, A. G., *Am. J. Med.*, **23**, 205-11 (1957)
271. Roberson, W. J., and Horvath, S. M., *Am. J. Physiol.*, **192**, 345-52 (1958)
272. Ohara, I., and Sakai, T., *Tôhoku J. Exptl. Med.*, **66**, 79-90 (1957)

273. Davis, J. O., Goodkind, M. J., and Ball, W. C., Jr., *Circulation Research*, **5**, 388-94 (1957)
274. Braunwald, E., Welch, G. H., Jr., and Sarnoff, S. J., *Circulation Research*, **5**, 539-45 (1957)
275. Wegria, R., Muelheims, G., Ireissaty, R., and Nakano, J., *Circulation Research*, **6**, 301-6 (1958)
276. Moscovitz, H. L., and Wilder, R. J., *Am. Heart J.*, **53**, 741-53 (1957)
277. Moscovitz, H. L., and Wilder, R. J., *Am. Heart J.*, **54**, 572-79 (1957)
278. Wégria, R., Muelheims, G., Golub, J. R., Ireissaty, R., and Nakano, J., *J. Clin. Invest.*, **37**, 471-75 (1958)
279. Welch, G. H., Jr., Braunwald, E., and Sarnoff, S. J., *Circulation Research*, **5**, 546-51 (1957)
280. Alexander, N., and Drury, D. R., *Am. J. Physiol.*, **191**, 476-80 (1957)
281. Alexander, N., Hinshaw, L. B., and Drury, D. R., *Circulation Research*, **5**, 375-78 (1957)
282. Franco, A., *Am. Heart J.*, **55**, 239-51 (1958)
283. Kuhn, L. A., Lasser, R. P., and Gordon, A. J., *J. Appl. Physiol.*, **11**, 161-68 (1957)
284. Wégria, R., Nakano, J., McGiff, J. C., Rochester, D. F., Blumenthal, M. R., and Muraviev, T., *Am. J. Physiol.*, **193**, 147-50 (1958)
285. Mascitelli-Coriandoli, E., Boldrini, R., and Citterio, C., *Nature*, **181**, 1215-16 (1958)
286. Sobel, H., and Graboyes, S., *Proc. Soc. Exptl. Biol. Med.*, **97**, 725-26 (1958)
287. Matejcek, E., Takac, M., and Stubnova, G., *Cardiologia*, **31**, 589-98 (1957)
288. Cabrera, G., Beuren, A., and Bing, R. J., *Circulation Research*, **6**, 15-19 (1958)
289. Szekeres, L., Lichner, G., and Varga, F., *Arch. Kreislaufforsch.*, **28**, 125-35 (1958)
290. Simmons, D. H., Hemingway, A., and Ricchiuti, N., *J. Appl. Physiol.*, **12**, 255-61 (1958)
291. Angelakos, E. T., Laforet, E. G., and Hegnauer, A. H., *Am. J. Physiol.*, **189**, 591-95 (1957)
292. Arnovljevic, V., *Cardiologia*, **31**, 420-25 (1957)
293. Berne, R. M., Jones, R. D., and Cross, F. S., *J. Appl. Physiol.*, **12**, 431-36 (1958)
294. Angelakos, E. T., Deutsch, S., and Williams, L., *Circulation Research*, **5**, 196-201 (1957)
295. Datey, K. K., Sen, P. K., and Shribastava, B. N., *Indian Heart J.*, **9**, 65-76 (1957)
296. Emslie-Smith, D., *Brit. Heart J.*, **20**, 175-82 (1958)
297. Santos, E. M., and Kittle, C. F., *Am. Heart J.*, **55**, 415-20 (1958)
298. Badeer, H., and Khachadurian, A., *Am. J. Physiol.*, **192**, 331-34 (1958)
299. Brendel, W., Albers, C., and Usinger, W., *Arch. ges. Physiol.*, **266**, 341-56 (1958)
300. Brendel, W., Albers, C., and Usinger, W., *Arch. ges. Physiol.*, **266**, 357-72 (1958)
301. Albers, C., Brendel, W., Hardewig, A., and Usinger, W., *Arch. ges. Physiol.*, **266**, 394-407 (1958)
302. Albers, C., Brendel, W., Hardewig, A., and Usinger, W., *Arch. ges. Physiol.*, **266**, 373-93 (1958)
303. Behmann, F. W., and Bontke, E., *Arch. ges. Physiol.*, **266**, 408-21 (1958)
304. Behmann, F. W., *Arch. ges. Physiol.*, **266**, 422-46 (1958)
305. Blatteis, C. M., and Horvath, S. M., *Am. J. Physiol.*, **192**, 357-63 (1958)

306. Hunter, J., *Can. J. Biochem. and Physiol.*, **35**, 605-13 (1957)
307. Szilagyi, T., Benko, K., and Csernyanszky, H., *Nature*, **181**, 909 (1958)
308. Hannon, J. P., and Covino, B. G., *Am. J. Physiol.*, **192**, 121-25 (1958)
309. Henneman, D. H., Bunker, J. P., and Brewster, W. R., Jr., *J. Appl. Physiol.*, **12**, 164-68 (1958)
310. Gilbert, J. L., Lange, G., Polevoy, I., and Brooks, C. McC., *J. Pharmacol. Exptl. Therap.*, **123**, 9-15 (1958)
311. Cotten, M. de V., Moran, N. C., and Stopp, P. E., *J. Pharmacol. Exptl. Therap.*, **121**, 183-90 (1957)
312. Katsh, S., *Am. J. Physiol.*, **188**, 538-42 (1957)
313. Lindell, S. E., *Acta Physiol. Scand.*, **41**, 255-60 (1957)
314. Oppenheimer, M. J., Lynch, P. R., and Ascanio, G., *Am. J. Physiol.*, **191**, 481-86 (1957)
315. Nahas, G. G., *Circulation Research*, **5**, 489-92 (1957)
316. Benforado, J. M., *J. Pharmacol. Exptl. Therap.*, **122**, 86-100 (1958)
317. Bennett, D. R., Andersen, K. S., Andersen, M. V., Jr., Robertson, D. N., and Chenoweth, M. B., *J. Pharmacol. Exptl. Therap.*, **122**, 489-98 (1958)
318. Sanyal, P. N., and Saunders, P. R., *J. Pharmacol. Exptl. Therap.*, **122**, 499-503 (1958)
319. Cotten, M. de V., and Stopp, P. E., *Am. J. Physiol.*, **192**, 114-20 (1958)
320. Caviezel, R., and Wilbrandt, W., *Helv. Physiol. et Pharmacol. Acta*, **16**, 12-21 (1958)
321. Smith, J. A., *Proc. Soc. Exptl. Biol. Med.*, **97**, 869-71 (1958)
322. Vick, R. L., and Kahn, J. B., Jr., *J. Pharmacol. Exptl. Therap.*, **121**, 389-401 (1957)
323. Gaffney, T. E., Kahn, J. B., Jr., Van Maanen, E. F., and Acheson, G. H., *J. Pharmacol. Exptl. Therap.*, **122**, 423-29 (1958)
324. Berman, D. A., Masuoka, D. T., and Saunders, P. R., *Science*, **126**, 746-47 (1957)
325. Rebar, J., Jr., Rebar, B. T., and Omachi, A., *Circulation Research*, **5**, 504-9 (1957)
326. Hess, M. E., and Haugaard, N., *Circulation Research*, **6**, 256-59 (1958)
327. Johnson, E. A., and Robertson, P. A., *Nature*, **180**, 1483-84 (1957)
328. Williams, E. M. V., *Lancet*, **I**, 943 (1958)
329. Holland, W. C., *Am. J. Physiol.*, **190**, 492-94 (1957)
330. Robertson, A. C., Foulks, J. G., and Daniel, E. E., *J. Pharmacol. Exptl. Therap.*, **122**, 281-94 (1958)
331. Blatteis, C. M., and Horvath, S. M., *Am. J. Physiol.*, **192**, 353-56 (1958)
332. Karczmar, A. G., Bourgault, P., and Elpern, B., *Proc. Soc. Exptl. Biol. Med.*, **98**, 114-18 (1958)
333. Swain, H. H., and Weidner, C. L., *J. Pharmacol. Exptl. Therap.*, **120**, 137-46 (1957)
334. Swain, H. H., and McCarthy, D. A., *J. Pharmacol. Exptl. Therap.*, **121**, 379-88 (1957)
335. Russell, F. E., Barritt, W. C., and Fairchild, M. D., *Proc. Soc. Exptl. Biol. Med.*, **96**, 634-35 (1957)

PERIPHERAL CIRCULATION¹

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During the two years since Peterson's review of the peripheral circulation in the *Annual Review of Physiology* (1), such a tremendous volume of literature on this subject has appeared that the references reviewed in the present paper have had to be selected with extreme care. Also, because the pharmacological aspects of the peripheral circulation were reviewed in the *Annual Review of Physiology* by Bovet & Carpi in 1957 (2), only those aspects of the pharmacology of the peripheral circulation that shed light on physiological mechanisms will be covered. This paper presents, first, the general dynamics of the peripheral circulation and then studies relating to certain peripheral circulatory diseases and regional vascular beds.

BLOOD VOLUME

Measurements.—The methods for measuring blood volume have been reviewed by Mertz (3). Especially interesting in the past two years has been the development of far more knowledge concerning the vascular-extravascular interchange of fluids and proteins (4 to 12), the lack of which has long been one of the major deterrents to accurate measurements of blood volume. Studies, particularly by Mayerson *et al.* (4, 5) and Huggins *et al.* (7 to 11), have shown that transfusion of large excesses of blood or other fluids into the circulation can sometimes so overstretch the capillaries that a "stretched pore phenomenon" (5) occurs, allowing excessive leakage not only of fluid into the extravascular spaces but also of large portions of the plasma protein. Because of this effect the usual dilution techniques for measuring blood volume become very inaccurate. However, by using radioactive protein, then allowing the plasma proteins to come to equilibrium with the extravascular proteins, and finally mathematically accounting for the extravascular portion of the proteins, it is possible to determine plasma volumes with reasonable accuracy even when proteins leak from the circulation very rapidly (4, 6).

Regulation of blood volume.—Sjostrand (13), in reviewing the regulation of blood volume, points out that the basic regulatory factor is the capacity of the vascular system itself. The next factor of importance is the shift of fluids through the capillary membrane between the extravascular and vascular spaces. Following transfusion into a normal animal, fluid loss into the tissues begins immediately and continues until the blood volume is readjusted to a normal value; and, conversely, following hemorrhage the dynamics of capillary equilibrium cause an immediate onset of fluid transfer into

¹ The survey of the literature pertaining to this review was concluded May 1, 1958.

the circulatory system from the extravascular spaces (14). Also, within twenty-four hours after a severe hemorrhage approximately half of the protein lost from the plasma will have been replaced by protein from the extravascular spaces, and the remainder of the lost plasma protein is replaced in another two to five days by rapid anabolic formation of new protein (4).

Sympathetic control of the circulation is a third factor of importance in the regulation of blood volume (15 to 20). It has long been known that almost any reflex that excites the sympathetic nerves, including the carotid sinus reflex, the Cushing reflex, or others can alter the output of urine by the kidneys and consequently help to readjust extracellular fluid volumes. However, during the past several years Henry *et al.* (15 to 18) have pointed out that excessive pressure in the left atrium promotes a particularly strong reflex which increases urinary outflow, thereby reducing the blood volume and making the left atrial pressure return to normal. Finally, it has become apparent that hypovolemia, whether it results from decreased blood volume or decreased extracellular fluid volume, can cause increased aldosterone secretion and consequently retention of sodium and water by the kidneys (21, 22).

Pressure-volume relationships.—Pressure-volume curves have been studied, especially in the limbs (23 to 26). In general, the sigmoid shape of these curves has been reaffirmed, and especially interesting has been the demonstration by Eckstein & Hamilton (26) that, though large volumes of blood normally accumulate in the vessels of dependent limbs, concomitant stimulation by epinephrine and norepinephrine keeps the blood in the central venous reservoir. It is suggested that this is of importance to prevent syncope when a person assumes the upright position.

CAPILLARY CIRCULATION

Zweifach (27) and Illig (28) have recently reviewed the behavior of the microcirculation. Illig pointed out that under normal experimental conditions no evidence of swelling or contraction of either the endothelial cells or Rouget cells of the capillaries can be discerned. Also, he has confirmed the presence of muscular "A-V bridges" or "central canals" in the rat mesentery, and likewise the presence of precapillary sphincters around the entrances of the capillaries leaving muscular vessels such as the arterioles or central canals.

Studies on arteriovenous anastomoses (29 to 34), including two new reports on the microsphere method for determining the number and sizes of shunts (33, 34), have indicated their presence in the muscles as well as in the skin. Also, specific cholinesterase has been found in the nerve fibers about the A-V anastomoses of the skin (30), a fact indicating that these structures have a cholinergic innervation.

Studies on capillary permeability have shown that the corticoids and ACTH (35, 36, 37) and a normal protein factor called protein "S" (38) have considerable ability to enhance capillary resistance against rupture; other studies in which excessive quantities of fluids containing macromolecular

particles were administered have shown that the capillary pore size can be greatly stretched when the vascular capacity is overloaded by excess blood volume (5). There is also evidence that calcium affects the capillaries to promote increased absorption while diminishing filtration (39), and a study on the gut employing perfusates with different colloid osmotic pressures has reconfirmed the quantitative aspects of Starling's law of the capillaries (40). Finally, it has been shown that potassium traverses the capillary walls at about twice the rate of sodium (41), an observation indicating that potassium has the ability to pass through the capillary wall in ways other than through the pores themselves.

Using purified protein fractions and appropriately prepared semipermeable membranes, Ott has determined the relative colloid osmotic pressures of the various plasma protein fractions (42). For 5 per cent solutions the measured colloid osmotic pressures of different purified fractions are as follows: α_1 globulins, 39 mm. Hg; albumin, 17 mm. Hg; α_2 globulin, 9 mm. Hg; beta globulin, 8 mm. Hg.; and gamma globulin, 6 mm. Hg.

An especially interesting study of the function of capillaries is that of Agostoni *et al.* (43) showing that the pulmonary capillaries in the visceral pleura exert a net absorption pressure through the pleura of about 15 mm. Hg. Presumably this results from the very low capillary pressure in the pulmonary system. A similar absorption pressure was not found for the parietal pleura. Thus, it appears that the negative intrapleural pressure is caused by the absorption pressure of the visceral pleura, and because this force is greater than the elastic retraction force of the lungs it holds the lungs against the chest walls. Of value also has been a new study on the permeability of the blood-brain barrier in which it has been shown that anoxia and almost any modification of tonus or vascular caliber of the cerebral plexus diminishes the permeability of the blood-brain barrier (44). Therefore, it is suggested that the capillaries of the cerebral plexus normally operate under almost optimal conditions. Using I^{131} albumin, Frick & Scheid-Seydel (45) have shown that cerebrospinal fluid albumin is derived almost entirely from plasma albumin, and that it takes about three days for iodinated albumin injected into the blood to reach a maximum in the cerebrospinal fluid.

Extracellular fluid volume.—The extracellular fluid will be reviewed in detail elsewhere in this volume by Selkurt, but several valuable contributions in this field related to the function of the circulation are the following. Critical studies on the methods of measurement of extracellular fluid volume (46 to 50) have again questioned the validity of thiosulfate and sucrose methods for giving even approximately accurate estimations. Comparative studies in nephrectomized dogs (46) showed that neither thiosulfate nor sucrose measures with accuracy a provoked 1 l. expansion of the extracellular fluid volume, whereas measurements with mannitol and radiosulfate do reflect the increment with fair accuracy. The regulation of extracellular fluid volume has been reviewed by Bresler (51) and Smith (52); they have reached the

general conclusion that no particular theory for volume regulation yet deserves complete respect.

Lymphatic flow.—Measurements on the flow of thoracic duct lymph (53, 54) indicate that the lymphocyte probably has a life as short as two hours in the blood stream, and measurements have shown that the rate of thoracic duct and right lymphatic duct lymph flow in the rabbit is approximately 2.1 ± 0.29 ml. per kg. per hr. (54). The function of lymphatics in local areas has also been studied, including the absorption of water from the intestines by lymphatic pathways (55) and the possibility that the lymphatics of the kidneys might act as a safety release valve in instances of swelling of the kidneys (56, 57). Of considerable theoretical importance also has been an anatomical study of lymphatics at the iridocorneal angle of the eye which has indicated that enough lymphatic pathways exist in the neighborhood of the canal of Schlemm (and possibly connect with this canal) to allow continual removal of proteins from the anterior chamber of the eye (58). An explanation for the almost zero concentration of proteins in the anterior chamber has long been a major problem, particularly because of lack of knowledge of the lymphatics draining this portion of the eye. Also, Langham (59) has reviewed the aqueous humor and the regulation of pressure in the eye.

CARDIAC OUTPUT AND BLOOD FLOW

Measurement of cardiac output.—New procedures employing indicator dilution techniques to measure cardiac output have received especial attention (60 to 71). The most interesting of these is based on the development of an improved continuous hemoglobin recorder by Lochner & dal Ri (64) which can record the dilution curve of arterial hemoglobin following intravenous injection of dextran in quantities as small as 2 to 4 ml. Repeated dilution curves can be made *ad infinitum* using nothing more than the injection of a clear solution. Also, Kramer & Ziegenrucker (71) have reported a new dye, "Rie 1743", that has sufficient absorption at 805 $m\mu$, an isobestic point of hemoglobin, so that concentration curves can be recorded regardless of the degree of hemoglobin oxygenation. Along with the development of new indicator dilution methods, critical studies have shown that indicator recirculation can frequently cause such marked abnormality of the indicator dilution curve that it is completely unreliable (72). Also measurements of cardiac output by means of pulse contour methods, using formulas proposed by Hamilton, Remington, Starr, and their associates, showed no accurate correlation with simultaneous measurements made by the Fick method (73).

An arteriovenous oxygen difference recorder has been described for use in following the cardiac output continuously by the Fick method (74).

Regulation of cardiac output.—Factors that regulate cardiac output have received especial attention in the last two years (75 to 98). Several studies have shown a high degree of correlation between temporary changes in blood volume and cardiac output (75 to 78), while others have shown that the degree of tone in the vascular system can greatly affect the cardiac output

(78 to 88). For instance, epinephrine injection enhances the tone of the venous reservoirs, thereby promoting considerably increased venous return (79, 80); and, similarly, eliciting the carotid sinus reflex usually increases the venous return as well as increasing the total peripheral resistance (83). On the other hand, drugs or physical states that cause venous dilatation result in diminished venous return and consequently depressed cardiac output (84 to 87). Likewise, decreased resistance in the peripheral circulatory system, such as that resulting from anemia (89, 90), exercise (91), arteriovenous fistulae (92, 93, 94), or oxygen lack (95), leads in every instance to enhanced venous return and consequently enhanced cardiac output.

Further developments in an overall theory for regulation of cardiac output have also been made (80, 96, 97), indicating that the peripheral circulation factors affecting cardiac output can be grouped into those that affect the mean circulatory pressure (such as blood volume, vascular tone, and tissue turgor) and those which affect the "resistance to venous return" (such as blood viscosity, vascular dimensions, and arteriovenous fistulae). An increase in mean circulatory pressure, whatever the cause, increases the tendency for venous return, while an increase in resistance to venous return, regardless of cause, reduces the tendency for venous return (96).

The effect of pressure breathing on venous return and cardiac output has been studied in new ways by Kaufmann & Marbarger (99) and Sarnoff and co-workers (100, 101). Counterpressure on the animal during positive pressure breathing helped to counteract the effects of the positive pressure as long as the pressure in the lungs was only 17 mm. Hg; but when it was increased to 26 mm. Hg, counterpressure proved to be of essentially no value (99). Various sympathomimetic drugs can also help overcome the effects of pressure breathing (100, 101), and these drugs can also counteract to a great extent the effects of cardiac tamponade (102). These findings are consonant with the general theory of overall cardiac output regulation which states that venous return is proportional to mean circulatory pressure minus right atrial pressure (96). When the right atrial pressure is elevated as a result of pressure breathing or cardiac tamponade, simultaneous elevation of the mean circulatory pressure by means of counterpressure or sympathomimetic drugs can overcome the elevated atrial pressure.

Gauer & Sieker (103), in studying the venous circulation in relation to cardiac function, have shown that central venous pressure can be recorded with reasonable accuracy from a vein in the right arm if the subject will lie on his right side with his right arm hanging down, and Duomarco *et al.* (104) have shown that the pulmonary veins, on leaving the lungs to enter the intrapleural space, are subject to the same collapse factors as the peripheral veins.

LOCAL BLOOD FLOW

Many different methods for measuring local blood flow have been developed, improved, or critically evaluated, including an indicator dilution method (105), the distribution of chemical substances to different portions

of the vascular bed (106, 107, 108), plethysmographic methods (109 to 112), a heat conductivity method (113), the thermostromuhr (114), the bubble flowmeter (115), rotameters (116, 117), blood velocity methods (118, 119), a circulation time recorder (120), and a mechanical recorder in which a rubber membrane is intermittently displaced by the flowing blood (121). Of particular interest are improvements made by Hilger & Brechtelsbauer (116) in the rotameter which are said to eliminate some of the coagulation and pulsation problems that occur with the Shipley rotameter.

Peripheral resistance.—The relationship of vascular pressures to the total peripheral resistance has received special attention (122 to 127), and all experiments have demonstrated that increased venous or arterial pressure, particularly venous pressure, decreases the peripheral resistance. The studies of Levy (122) indicate that so-called "anomalous blood viscosity" can be adequately explained by passive distention of the vascular bed at progressively higher and higher pressures. Folkow & Lofving (126) found that the distensibility of the "resistance" vessels of the systemic circulation is considerable at low degrees of vasomotor tone but minimal at high degrees.

The concept of critical closing pressure has again received considerable comment (122 to 128). In Levy's studies on the entire hind limb of a dog, no distinct critical closing pressure could be found (122, 123) and most of the observations could be explained by a gradual decrease in vasodilatation with falling pressures rather than the occurrence of an abrupt critical closing pressure. Similarly, in Folkow's studies a satisfactory critical closing pressure could be elicited only under very special conditions (126). On the other hand, Roddie & Shepherd in studying digital resistance in relation to transmural pressure found a very distinct critical closing pressure in the finger (127). It is possible, therefore, that the critical closure concept applies only to certain vascular beds rather than to all of them, or that the failure to find a distinct critical closing pressure in some portions of the circulation might be caused by different levels of critical closing pressures in different vessels so that no distinct pressure can be found at which flow suddenly becomes zero.

Haddy and his associates (129 to 133) and Kelly & Visscher (134) have continued to use microcatheterization methods for studying resistance changes in minute vessels of different organs of the body, showing among other interesting results that an increase in venous pressure either in renal (129) or foreleg veins (133) often causes a weak "venous-arteriolar" reflex. On the other hand, Roddie & Shepherd (127) found that increased venous congestion causes passive dilatation of the resistance vessels of the fingers, thus providing evidence against the thesis of a venivasomotor reflex, at least in the digital area of the circulation.

In a mathematical study, Maude & Whitmore (135) have shown that variations of blood viscosity with tube diameter can be explained on the basis of a layer of low viscosity fluid adjacent to the wall. By analyzing results published by others of the variations of viscosity with tube diameter, it was demonstrated that the drop in red cell concentration and the difference

in velocity of the red cells versus plasma in narrow tubes can be explained by mechanical displacement of the cells away from the wall.

An interesting method for estimating changes in peripheral resistance has been to occlude an artery suddenly while continuously measuring the pressure beyond the occlusion point (136, 137). The slower the rate of pressure decline after occlusion, the greater is the resistance assumed to be. Using this method vascular resistance was found to be considerably greater in hypertensive patients than in normals (136).

The reactive hyperemia mechanism for regulating local blood flow and local resistance has received especial emphasis (138 to 150). Golenhofen & Hilderbrandt (138) found the extra blood flow that occurs in muscle during a period of reactive hyperemia to be approximately proportional to the "blood flow debt" that has occurred during the period of occlusion. Also, these authors found that the reapprach of blood flow to the control value after a period of reactive hyperemia is usually characterized by two or three damped oscillations in blood flow above and below the control value, thus indicating the presence of a negative feedback control system. Patel & Burton (139), in studying reactive hyperemia of the finger, showed that the calculated "blood flow debt" on the average was approximately 51 per cent repaid, but this was sufficient to satisfy completely the "nutritional debt" of the skin.

Several attempts have been made to explain the basic mechanism of the increased blood flow during reactive hyperemia (143 to 149). Lambert showed that maintenance of the endovascular pressure of ischemic muscles at a constant value by infusion of saline or dextran did not modify the reactive hyperemia reaction (143, 144, 145). Also, in contradistinction to the results of Golenhofen (138), he found a very great inconstancy of repayment of the "blood flow debt", for which reason he believes that the accumulation of metabolites is not the determinant of the vascular response. Therefore, he has suggested that a vasoconstrictor substance might normally be produced elsewhere in the body but constantly inactivated by the peripheral vessels. During ischemia, this vasoconstrictor substance theoretically would become almost totally inactivated so that the vessels would then become greatly dilated. Other studies, on the other hand, indicate that blood flow in the coronary system at least is determined almost entirely by the rate of cardiac oxygen consumption (146, 147) except when the heart is greatly overloaded, in which case other factors besides oxygen debt can then cause prolonged increase in blood flow (147).

Thorban & Schonbach (150), in studying the effect of oxygen emboli in the ear arteries of the rabbit, showed that a small amount of oxygen passes on into the veins immediately, but then blood flow stops for 7 to 12 min. before resuming.

ARTERIAL PRESSURE AND HEART WORK

Measurement.—Several new methods for measuring arterial pressure and pressures in small blood vessels have been described, including chronic

catheterization of vessels (151), a microchamber method for measuring lateral pressure in small blood vessels (152), and several indirect pressure measuring devices for human beings or smaller animals (153, 154, 155). Stegemann has described an especially interesting method for recording the arterial pulse pressure contour without puncturing an artery (155). A small and very rigid chamber with one open side is placed over the radial artery; then the edges of the open side of the chamber are made to fit snugly against the skin by heating paraffin around the chamber edges and allowing the paraffin to resolidify. A rubber bag inside the chamber is then filled with a sufficient amount of fluid to compress the radial artery until it no longer has any elastic tension. In this state the pressure in the artery is transmitted faithfully into the fluid of the rubber bag and from here to an appropriate transducer for recording the pulse pressure contour continuously.

Theoretical and applied studies with the ballistocardiograph have continued to receive the attention especially of Starr (156, 157) and Klensch (158 to 162) and their co-workers, and attempts are still being made to measure cardiac stroke volume and heart work from the ballistocardiogram, though the reliability of this procedure is very questionable.

Nervous regulation of arterial pressure.—A number of investigators have considered the effects of central nervous system vasomotor centers on arterial pressure (163 to 170), and Oberholzer (163) has reviewed the circulatory centers in the medulla. Several workers have evidence that a special cholinergic vasodilator system might exist, beginning in the motor cortex and passing through the mesencephalon and lateral horn cells of the spinal cord into the sympathetic nerves (164, 165, 166). However, in contrast to these studies, Langford *et al.* (167) found that stimulation of the motor cortex causes very marked and rapid increase in total peripheral resistance, with concurrent elevation of the systemic arterial pressure. It is difficult, at present, to reconcile these two opposite findings, though both of the mechanisms could possibly be present, one of them increasing the blood flow to the muscles during muscular exercise while the other increases the systemic arterial pressure. Fronkova *et al.* (168) showed that autonomic responses can be induced in dogs in response to conditioned reflexes, and during active stoppage of these reflexes an occasional animal develops severe degrees of autonomic excitation manifested by arterial pressure elevation, pulse rate increase, and acceleration of respiration. These are believed to be similar to the autonomic reactions that frequently occur in maladjusted human beings. Bock and co-workers (169) studied the effect of artificial fever on muscle blood flow in human beings and, contrary to what might have been expected, found that fever hardly affects muscle blood flow even though it causes considerable vasoconstriction in the skin. Tenney (170), in studying the potency of carbon dioxide as a stimulus of the vasomotor centers in the cat, found that even in spinal animals carbon dioxide in high enough concentration can cause extreme stimulation of the sympathetic system, resulting in marked secretion of catechol amines into the circulatory system. This

indicates that the spinal centers are considerably more susceptible to direct activation than has been assumed in the past.

The effects of reflexes, especially pressoreceptor reflexes, on the regulation of arterial pressure have received considerable attention (171 to 181). Brind *et al.* (172) showed that carotid sinus hypotension causes very little effect on cardiac output but elevates the systemic arterial pressure, principally as a result of increased total peripheral resistance. Warner (176) has used an electric analogue to increase the amplification of the carotid sinus reflex, in this way demonstrating that the lag time for changes in cardiac output following stimulation of the carotid sinus is considerably less than the lag time for changes in total peripheral resistance. Therefore, he suggests that the carotid sinus buffers sudden variations in arterial pressure, principally by changing cardiac output. Studies by Hilton & Brown (177) demonstrated that the maximum attainable arterial pressure following single injections of epinephrine is approximately the same after pressoreceptor denervation as before denervation. This was the conclusion to be expected, since the pressoreceptors, however active they might become, are unable to reduce sympathetic tone when a maximal dose of epinephrine is present in the circulating blood.

Brown (182) has reinvestigated the reflex effects of acutely elevated intracranial pressure with conclusions supporting Cushing's original observation that vasoconstriction is the dominant effect. Mukherjee (183, 184) has shown that bladder distention initiates a cord reflex that is transmitted downward through the splanchnic nerves to cause renal vasoconstriction and also to elevate arterial pressure. It is possible that renal vasoconstriction caused in this way, particularly if the reflex proves to be transmitted contralaterally, might be a cause of hypertension in unilateral pathological conditions of the kidneys.

Experiments on the effects of sympathetic activity on the peripheral circulation have shown that stimulation with frequencies as low as 10 cycles per sec. usually causes maximal effects on the circulation (185, 186, 187). Analysis of vasodilatation mechanisms in the ear vessels of the rabbit by Capon (187, 188) has demonstrated that by far the most important vasodilatation mechanism is release of sympathetic vasoconstriction, though evidence for a weak antidromic, noncholinergic type of vasodilatation was also obtained. In comparative studies of the effects of insulin hypoglycemia and of epinephrine and norepinephrine, DiSalvo *et al.* (189) have observed that insulin hypoglycemia causes almost identically the same peripheral circulatory effects as epinephrine and norepinephrine and, furthermore, that insulin hypoglycemic effects are blocked by administration of autonomic blocking agents. Finally, Lofving & Mellander (190) have shown in the cat that sympathectomy hardly changes the basal tone of the blood vessels in the skeletal muscles, while the blood vessels of the paw, with its abundance of arteriovenous anastomoses, become almost maximally dilated. This indicates that the skin vessels are controlled almost entirely by the

nervous system while the vessels of the muscles are probably controlled principally by local intrinsic chemical factors.

The effects of sympathetic reflexes on the venous system have been studied in many different ways, such as by measuring pressures in isolated venous areas (191, 192), measuring pressures in balloons placed in the veins (193, 194), and by microscopic observation of the small veins (195). In all instances venomotor tone was found to be increased by sympathetic activity, a fact illustrating that the venous system, probably fully as much as the arterial system, contributes to cardiovascular reflex responses.

Among the many studies on the functions of epinephrine and norepinephrine in relation to the peripheral circulation (196 to 213) have been demonstrations that pressor responses to single injections of these drugs become greatly intensified in spinal animals (196, 197), that physical activity markedly increases the excretion of both epinephrine and norepinephrine in the urine (199), that norepinephrine is synthesized by sympathetic nerves and ganglia in preference to the synthesis of epinephrine (201), and (in support of previous studies) that the vasodilator effect of epinephrine in skeletal muscle is caused by an increase in metabolism (204). The pharmacological effects of epinephrine, norepinephrine, and other drugs on the peripheral circulation have been reviewed by Bovet & Carpi (2) to which review the reader is referred. Besides the work noted above on epinephrine and norepinephrine, some of the representative studies in this field have concerned the circulatory effects of 5-hydroxytryptamine and other drugs of similar chemical composition (214 to 218), of different anesthetics (219, 220), of histamine and histaminase inhibitors (221, 222, 223), ganglionic blocking agents (224 to 227), and other hypotensive drugs (228, 229). In addition to these investigations, the actions of 5-hydroxytryptamine have recently been reviewed in detail by Page (230) and Lewis (231), and the hemodynamic effects of ether, cyclopropane, and barbiturates have been reviewed by Faulconer & Patrick (232).

Pulse pressures.—A number of different investigators have made additional studies on the genesis of the pulse pressure contour (233 to 241) and on the pulsatile changes in blood flow (242, 243). Also, methods have been devised for recording vascular wall tension (244) and vascular wall diameter (245), and the effect of a sympathomimetic agent metaraminol (Aramine), on length-tension curves of vascular walls has been studied (246). Especially important have been several analyses of the arterial tree as a resonant system. Warner (236), using an electric analogue consisting of a filter network designed to duplicate the resonant frequency and damping coefficient of an arterial segment, demonstrated that the general principles of a resonant circuit can be used to explain the distortion of a pressure wave as it is transmitted through an artery. Randall & Stacy (237) showed that the resonant frequency of the arterial system of the dog is approximately 180 cycles per min., and, because of this resonance, pressure pulses of this frequency are transmitted through the system with less impedance than pressure pulses of other frequencies. In a study on pulsatile flow, Spencer & Denison (242)

demonstrated that changes in the pressure contour "lead" the changes in flow rate by a phase angle of sufficient magnitude that inertia rather than resistance appears to play the dominant role in the genesis of the aortic flow pulse; therefore, the acceleration of the blood is almost proportional to the differential pressure pulse.

ABNORMALITIES OF THE PERIPHERAL CIRCULATION

Hypertension.—Considerable literature attempting to explain the basic causes of hypertension has again appeared recently (247 to 271). In studies on renoprival hypertension, Muirhead *et al.* (247) have shown that the elevation of pressure can be potentiated by addition to the diet of excessive protein, alanine, pyruvate, or lactate. Grollman (248) has been able to produce renal hypertension in both rats and dogs by either potassium or choline deficiency, and these effects can be further accentuated by substituting 1 per cent sodium chloride for the animal's drinking water or by administering alcohol. Crandall and co-workers (249) have restudied the problem of hypertension resulting from disturbances of the carotid sinus area. Of particular significance has been the demonstration in dogs that mechanical stricture of the carotid sinus areas, without actual occlusion of the carotid vessels, causes even more significant and more persistent hypertension than extirpation of the carotid sinus areas. It is presumed that this elevation of pressure results from failure of the pressure inside the vessels to stretch the pressoreceptors, thereby nullifying the normal inhibitor effect on the vasomotor center by the pressoreceptors. Culbertson *et al.* (250) have observed that the hemodynamic pattern of human hypertension in coarctation of the aorta is quite different from that observed in essential hypertension or renal hypertension. For instance, evidence for renal ischemia was found in only one of eight such patients, except in those in congestive heart failure, and there was no evidence of generalized vasoconstrictor influence throughout the body.

The renin mechanism (251 to 255) has again received much attention, and especially important has been the demonstration that hypertensin exists in two forms: hypertensin I and hypertensin II (251 to 254). Hypertensin I normally has no constrictor effect on isolated strips of blood vessels but, when activated by "hypertensin converting enzyme" [which has been prepared in a semipurified form from horse plasma (252)] into hypertensin II, it exerts the usual vasoconstrictor influence. However, extremely discouraging to the proponents of the renin theory of renal hypertension has been the demonstration by Schapiro *et al.* (255) that renin injection superimposed to the point of tachyphylaxis on hypertension caused by constant intravenous infusion of a renin solution always causes the arterial pressure to return to normal, though renin tachyphylaxis never lowers the arterial pressure of renal hypertensive animals, thus indicating with little room for doubt that renin hypertension and renal hypertension are entirely different entities.

New studies have been added to an already large volume of literature

indicating that vascular reactivity of the resistance vessels is enhanced in some types of hypertension (255 to 259). Redleaf & Tobian (260), however, showed that after inhibiting spontaneous tone in aortic strips cut from rats the response to norepinephrine was almost exactly the same in strips from hypertensive as from normotensive rats, and their conclusion was that increased vascular reactivity in hypertension might be caused by an altered "baseline" state rather than by hyperresponsiveness. Another factor that has been shown to modify the results in studies on renal hypertension is urea retention, for Skelton (261) has shown that administration of urea to rats as 20 per cent of their diet can cause such a hypotensive effect that hypertension sometimes completely fails to appear in otherwise hypertensive situations.

The peripheral circulation in congestive heart failure.—Several different studies have reaffirmed the fact that blood volume is usually elevated in congestive heart failure (272, 273, 274), and in each of these studies treatment has been accompanied by a statistically significant diminishment in blood or plasma volume. Also, the methods for measuring blood volume in patients with congestive failure have been critically analyzed, with the conclusion that T-1824 is quite adequate for measuring plasma volume (272) and that the most reliable method for measuring total blood volume is simultaneous use of P³² tagged cells and T-1824 dye (273). Electrophoretic studies by Decortis (275) in patients during the stage of decompensation showed a distinct diminution of blood albumin and a simultaneous elevation of the albumin to globulin ratio in the interstitial fluid, thus indicating that albumin is probably lost into the interstitial fluid during the phase of decompensation.

Davis and co-workers (276 to 283) have amassed considerable data indicating an increased aldosterone secretion by the adrenal glands in dogs with either right heart failure or thoracic inferior vena cava constriction. The difference between control and experimental preparations was very striking, with an average rate of aldosterone secretion, estimated from adrenal vein assays, of 2.7 $\mu\text{g. per hr.}$ in normal dogs, 11.6 $\mu\text{g. per hr.}$ in dogs with cava constriction, and 16.5 $\mu\text{g. per hr.}$ in dogs with heart failure (277). Also, their work has shown that hypophysectomy reduces aldosterone secretion in dogs with thoracic inferior vena cava constriction but does not inhibit it entirely; in these same animals it was found that the two layers of the adrenal cortex become markedly atrophic after hypophysectomy but the outer layer remains intact (279). In contrast to these studies, however, has been the finding by Driscoll *et al.* (284) that no significant difference in adrenal vein aldosterone could be detected between normal dogs and dogs with experimental congestive failure. Furthermore, aldosterone was not detected by chromatography or bioassay in three-day urine samples, either before or after heart failure was induced. These authors suggest, therefore, that increased secretion of aldosterone is not at all necessary for salt and water retention in congestive heart failure.

Jacobson *et al.* (285), using the thoracic inferior vena cava occlusion method, also produced congestive symptoms and found that occlusion of the vena cava causes almost immediate and sustained retention of sodium by the kidneys; release of the obstruction allows immediate return of sodium excretion. Also, studies by Underwood *et al.* (286) showed that acute cardiac tamponade causes an almost immediate increase in the plasma volume even before hormonal factors could possibly operate. These results are in accord with the theory that acute failure of the heart tends at first to reduce capillary pressure rather than to raise it—that is, that diminished flow of blood from the arteries into the capillaries has a more significant effect on capillary pressure than back pressure from the veins. These results support the theory that generalized edema does not occur in congestive failure until after retention of water and salt by the kidneys has taken place.

Alexander (287) in studying the development of congestive heart failure in rabbits following aortic constriction has shown that the effective circulating plasma volume increases early after the constriction and that this is associated with sodium retention by the kidneys and expansion of extracellular fluid volume. Further, constriction of the aorta in steps rather than sudden severe constriction prevents the congestive phase of failure in these animals (288). Foldi *et al.* (289) have shown that hypoxia alters kidney function in otherwise normal animals exactly as does decompensation, and they have evidence that elimination of hypoxia in cardiac decompensation displaces kidney function in the direction of normality. Therefore, he suggests that the abnormalities of kidney function in congestive heart disease might result from hypoxia rather than from pressure or hormonal alterations. Wood & Wilkins (290) have shown that the degree of venous tone is very great during cardiac decompensation but that this decreases considerably as the patient improves. This finding supports observations by many previous workers that increased sympathetic activity throughout the body [resulting in increased mean circulatory pressure (96), among other things] is an extremely important factor in preventing death of a patient with a weakened heart.

Circulatory shock.—The factors that make an animal resistant to shock have been studied in a number of different ways (291 to 296). Manger *et al.* (291) have shown that bleeding an animal an amount less than that required to produce shock causes only very slight increase in the plasma concentration of pressor amines, but bleeding to shock levels increases the pressor amines, especially epinephrine, as much as sevenfold or more, and anaphylactic shock frequently increases their level as much as tenfold or more. Farrell *et al.* (292) have shown that significant blood loss from an animal also results in marked increase in the adrenal secretion of aldosterone. Jacob and co-workers (293) have investigated the well-known protective action of dibenamine in hemorrhagic shock in the dog and conclude that this protection results mainly from a reduction of the bleeding volume required to institute the shock. With similar degrees of blood loss, the non-

dibenamized dog can tolerate shock better than can the dibenamized dog.

Crowell *et al.* (294) have shown in a large series that a dog with a hematocrit of approximately 35 per cent exhibits far greater resistance to the development of irreversible hemorrhagic shock than does a dog with either higher or lower hematocrit. They believe that with higher hematocrits the depressed blood flow caused by increased viscosity is detrimental to the animal and that with lower hematocrits depressed transport of oxygen is detrimental. Crowell & Smith (295) have been able to revive dogs completely after 15 min. of circulatory arrest by treating the dogs with a fibrinolytic activator before inducing the arrest. The activated fibrinolysin presumably prevents the development of minute clots throughout the circulatory system. Because recovery of function by the brain following ischemia is one of the most important problems in circulatory arrest or shock, Hirsch, Euler & Schneider (296) have studied the recovery of the brain following complete ischemia in the isolated head of a cat. The function of the brain, as judged by spontaneous action potentials, recovered completely in approximately 10 min. following 1 min. of ischemia, but following 10 min. ischemia the brain did not develop spontaneous action potentials until 35 to 120 min. later, and never recovered completely.

Attempts to explain the development of irreversibility in shock have again proved to be relatively fruitless (297 to 302). However, the following observations have been made. Shocked animals in which normal intestinal blood flow was maintained by a special perfusion system showed greater resistance to development of irreversibility than did animals without intestinal perfusion (297). By continuous recording of intestinal weight changes during the development of shock, Johnson & Selkurt (298) have shown that intravascular pooling of blood in the intestines is not a necessary feature of hemorrhagic shock. And in animals with oligemic shock both the systemic (301) and pulmonary (302) circulatory resistances have been shown to be decreased.

In anaphylactic shock White & Woodard (303) have shown that moderate quantities of heparin are released into the thoracic duct, but these quantities often are not sufficient to elevate the heparin levels in the blood. Lecomte (304), on the basis of comparisons between histamine shock and anaphylactic shock, has concluded that the hypotension in rabbits caused by anaphylaxis results, first, from pulmonary arterial vasoconstriction and, second, from systemic vasodilatation resulting directly from the antigen-antibody reaction. Also, he has shown that increased excitability of the vasomotor center helps to protect against anaphylactic shock, while diminished excitability provokes more intense shock (305).

Hypothermia.—The effects of hypothermia on the cardiovascular system have been reviewed by Brown (306), and new studies have confirmed previous observations that, at least in the initial stages of hypothermia, the arterial pressure is maintained at a high level while the cardiac output, the pulse rate, and general metabolic functions decrease (307 to 310). Lynch &

Adolph (310), using microscopic methods for studying rats and hamsters during hypothermia, found that the increased total peripheral resistance occurring in hypothermia does not result from vasoconstriction but, instead, from increased blood viscosity. In an attempt to establish optimal temperature ranges for maintenance of circulatory arrest under hypothermia, Hirsch *et al.* (311) studied the ability of the brain to recover from complete ischemia in animals at different temperatures. Between 23° and 27°C. the latency period for reappearance of spontaneous cerebral action potentials was the shortest. At lower temperatures the brain would not recover well simply because of the low temperatures, and at higher temperatures increased damage presumably occurred in the brain during the ischemia which prevented early reappearance of spontaneous action potentials.

In studying cold acclimatization Brown (312) concluded that Eskimos resist cold stress better than residents of temperate climates principally because of the enhanced ability of the arteriovenous anastomoses supplying the heat exchange mechanism in their skin to constrict. Covino & Beavers (313) have found that exposure of dogs to subarctic temperatures for periods as long as four weeks seemingly causes complete acclimatization. Imig, Roberson & Hines (314) have shown in dogs with frozen limbs that the blood flow following thawing becomes greater than normal in an unsympathectomized limb, but in a sympathectomized limb returns only to control values. This indicates that the increased blood flow might be caused by damage to the vasoconstrictor nervous system rather than by a direct effect on the vessels themselves.

REGIONAL BLOOD FLOW

Pulmonary circulation.—The effects of various mechanical factors on the pulmonary circulation have received much attention. Carlill & Duke (315), Borst *et al.* (316), and Piiper (317) have all shown that elevation of the pulmonary vascular pressure, particularly left atrial pressure, reduces the pulmonary vascular resistance. Carlill & Duke point out especially that elevation of the left atrial pressure in the lower pressure ranges (up to 15 cm. of saline) causes very marked decrease in pulmonary resistance but elevation above this value has little effect on the resistance, a fact illustrating that many collapsed vessels probably open up in the lower pressure ranges. Burton & Patel (318) have studied the effects of inflating the lungs by means of negative pressure outside the lungs versus positive pressure inside the lungs, showing that negative pressure inflation always causes the pulmonary resistance to decrease, while positive pressure inflation causes decreased resistance in the lower pressure ranges but increased resistance in the higher pressure ranges. The negative pressure effects can be explained on the basis of simple distention of the vessels, while the positive pressure effects in the low pressure range can possibly be explained by the straightening out of vascular kinks and in the high pressure range by compression of the vessels. Harrison and co-workers (319), on studying the acute and chronic effects of extirpation

of 75 to 85 per cent of the functional lung, showed that the pulmonary arterial pressure increased immediately approximately 100 per cent, but six to seven years later the pulmonary arterial pressure had fallen to only 17 per cent above the preoperative values. Several workers studying the effects of unilateral occlusion of a pulmonary artery either in man or animal (320 to 325) have found only slight increases in pulmonary arterial pressures and otherwise essentially normal respiratory function. This method is of special value, though, for predicting the ability of a patient to withstand unilateral pneumonectomy (321). DuBois & Marshall (326) studied pulmonary capillary blood flow throughout the respiratory cycle using an indirect nitrous oxide absorption technique, but they found no significant changes during the normal respiratory cycle.

Cournand has reviewed the control of the pulmonary circulation in man (327), and other workers have investigated specific facets of the reflex regulation of pulmonary resistance. Borst, Berglund & McGregor (328) have compared the effects of epinephrine, norepinephrine, and 5-hydroxytryptamine on the pulmonary circulation of the dog and found that all of these exert a vasoconstrictor action but that 5-hydroxytryptamine has the most marked effect. Patel & Burton (329) have shown that active vasoconstriction in the lungs of rabbits, following administration of different vasoconstrictor drugs, can be demonstrated by injecting vinyl acetate, which immediately forms casts of the small pulmonary arteries which can be studied later by microscopic methods. Others have shown that acetylcholine can cause vasodilatation both in man and in animals (328, 330, 331), though Rose (331) noted that extreme doses of acetylcholine can increase the pulmonary vascular resistance in contrast to the vasodilating effect of small doses.

Effects of hypoxia and hypercapnia on pulmonary resistance have been restudied by Borst and co-workers (332), who found that both of these usually promote vasoconstriction beginning within one-half minute but not reaching a maximum until 8 to 20 min. later. However, in some animals no effect could be discerned until 6 to 8 hr. after the induction of anesthesia. On the other hand, Aviado *et al.* (333) in studying anoxia and Peters (334) in studying hypercapnia observed very variable results, indicating that hypoxia and hypercapnia probably do not have strong vasoconstrictor effects if any at all.

De Burgh Daly and co-workers studied effects of pressoreceptor (335, 336, 337) and chemoreceptor (338) stimulation on pulmonary vascular resistance. Pressoreceptor stimulation invariably caused an increase in resistance, while chemoreceptor stimulation invariably caused a decrease in resistance. The pressoreceptor effect on the resistance was not affected by blocking either sympathetic or parasympathetic nerves to the lungs, for which reason it was assumed that the pulmonary vascular effects occur entirely secondarily to direct effects on the heart and systemic circulation. On the other hand, section of the vagi blocked the pulmonary vasodilator effect of the chemoreceptors, indicating that a cholinergic reflex is the possible mediator of this effect.

Ferri and co-workers (339) and Courtoy & Salonikides (340) have studied local reflexes within the pulmonary system. Ferri's studies indicated that constriction of the pulmonary veins causes reflex arteriolar constriction in the lungs mediated by way of the sympathetic nervous system and blocked by ganglionic blocking agents. Courtoy's studies indicated, contrary to many previous claims by others, that diffuse pulmonary embolism causes no reflex vascular spasm in the lungs but elevates the pulmonary arterial pressure strictly as the result of mechanical effects.

Fluids of the lungs, especially in relation to pulmonary edema (341 to 349) and pulmonary blood volume (350 to 353) have received special study. Visscher *et al.* (341) have reviewed the physiological and pharmacological aspects of pulmonary edema, while Luisada and co-workers (342, 343) have shown that the pulmonary edema resulting from powerful stimulation of the vasomotor center of the central nervous system is probably caused by excessive shift of blood from the systemic to the pulmonary circulation. Hawthorne *et al.* (344) have shown that acute pulmonary edema can be produced by simultaneous creation of aortic insufficiency and renal artery constriction, but that this type of pulmonary edema can be prevented by prior construction of an atrial septal defect (345). Borst *et al.* (346) have demonstrated that the fluid changes occurring in the lungs are not sufficient at left atrial pressures below 30 to 40 cm. of water to cause marked changes in the compliance curves of the lungs, though above these values moderate effects on compliance were observed. Studies by Fishman and co-workers (348) showed that the sodium and chloride spaces of the lungs are large in comparison with those of other tissues, for which reason it is believed that large amounts of fluid might line the inner alveolar walls.

Some miscellaneous effects related to pulmonary blood flow have been the demonstration in isolated dog lungs that the blood flow through arteriovenous anastomoses amounts to about 1.6 per cent of the entire blood flow (354); that immediately after beginning exercise the pulmonary arterial pressure rises, but on continued exercise the pressure often falls to values even below the control resting levels (355); and that in old age the volume distensibility of the pulmonary arteries decreases to as low as 28 per cent of normal, but because of simultaneous dilatation of the pulmonary arterial system the overall distensibility remains approximately unchanged (356). Finally, it has been shown that institution of blood flow delay from the lungs to the brain by placing a blood reservoir between the heart and the brain can cause spontaneous Cheyne-Stokes breathing in dogs (357). The delay presumably allows increased time for gaseous changes to occur in the lungs, body fluids, and respiratory center during the respective apneic and ventilatory phases of respiration, thereby greatly intensifying the feedback responsiveness of the respiratory control system and resulting in spontaneous oscillation.

Cerebral circulation.—Carlyle & Grayson (358), using a thermal conductivity method, have made an extensive study of the factors that control cerebral blood flow. Especially have they found that ganglion-blocking

agents hardly affect resting blood flow and that a fall in arterial pressure down to a level of approximately 40 mm. Hg causes little if any change in cerebral blood flow, because of an automatic decrease of unexplained origin in intracerebral vascular resistance. Finnerty *et al.* (359), on the other hand, found that administration of hexamethonium to a patient tilted into the head-up position always causes both a fall in cardiac output and also a proportionate fall in cerebral blood flow, thereby leading to cerebral ischemia. Green & Denison (360) in studying isolated beds of the cerebral circulation found, in contrast to the effects in almost all other circulatory beds, essentially no vasomotor response to epinephrine and arterenol.

Munck & Lassen (361) have found krypton 85, an inert gas, to be equally as satisfactory as nitrous oxide for measuring cerebral blood flow. Holmes *et al.* (362) have shown that the carotid blood in a cat supplies the cerebral hemispheres, the thalamic regions and part of the upper cerebellum, pons, and medulla while the lower medulla and cerebellum are supplied by vertebral blood. Whisnant *et al.* (363) have shown that ligation of both carotids and both vertebrals in the dog rarely causes death because of a large anastomotic circulation between the costocervical and omocervical arteries that join muscular branches of the vertebral arteries beyond the ligated point.

Splanchnic and hepatic circulations.—In two separate studies, the splanchnic blood volume has been found to be 21 and 21.7 per cent of the circulating blood volume respectively (364, 365). Acute ligation of the portal vein (366) causes the portal venous pressure to rise to a mean of 29 cm. H₂O almost immediately and then to fall during the ensuing 30 to 60 min., and the systemic arterial pressure falls within 10 to 20 min. to a level of 30 to 40 mm. Hg (367), remaining there until immediately prior to death. The blood volume of the extraportal portion of the circulation after portal occlusion diminishes by approximately 45 per cent before death occurs (367), indicating a tremendous shift of whole blood into the splanchnic area. In studying pressure-flow relationships in the splanchnic area, Selkurt *et al.* (368) found in denervated intestinal loops of the dog that blood begins to flow when the arterial pressure rises above 16 mm. Hg and increases more and more rapidly up to pressures as high as 240 mm. Hg, exhibiting a curvilinear relationship between pressure and flow with the convexity toward the pressure axis. Trapold (369) has shown that when the perfusion pressure to the mesenteric artery is maintained at a constant level, mesenteric resistance decreases markedly following the injection of ganglionic-blocking agents. In studies on the exchange rate of proteins between ascitic fluid and the serum, Schwartzkopff & Pickert (270) showed that Evans Blue injected into the ascitic fluid gradually diffuses into the blood so that by approximately the ninth day the concentration of Evans Blue in the ascites and in the plasma is nearly equal. Pressures obtained by splenic manometry (371, 372, 373) have proved to be an accurate measure of portal pressure.

In studies on liver blood flow, critical analysis of flow measurements by

heat conductivity determinations showed that a linear relationship between heat conductivity and blood flow does not exist (374) but, nevertheless, adequate calibration of the method can be attained. Using this method, spontaneous rhythmic fluctuations of about 20 per cent of the mean perfusion rate with a periodicity of close to 30 sec. were observed (375). It is possible that these fluctuations are a manifestation of the vasomotor waves seen elsewhere in the circulation. Horvath *et al.* (376) showed that animals continue to live following hepatic arterial ligation for periods up to 534 days without excessive damage to their livers, indicating that the portal venous blood supply is adequate to maintain almost normal liver function. In studying the factors that determine the rate of liver blood flow, Grayson & Mendel (377) demonstrated that at normal perfusion pressures about 30 per cent of the total liver blood flow is contributed by the hepatic artery and that most of the remainder comes from the gastrointestinal system. At these same pressures almost no blood flows from the spleen into the liver, but at low perfusion pressures as much as 23 to 60 per cent of the total liver flow comes from the spleen. In studying pressure-flow relationships in the liver, Reecker (378) demonstrated that increasing the perfusion pressure causes progressively less resistance to flow through the liver, presumably because of progressive stretching of the vessels.

Coronary circulation.—Sarnoff and co-workers (379) have demonstrated that the primary determinant of oxygen utilization by the myocardium is the total tension developed by the heart during contraction and, in turn, the myocardial oxygen consumption is the main determinant of coronary blood flow, though changes in aortic pressure and cardiac output can to a much smaller extent also affect coronary flow (380) even when there is no change in oxygen consumption. Sabiston & Gregg (381) demonstrated that sudden asystole causes immediate increase in flow through the coronary arteries and coronary sinus, thus demonstrating that under normal circumstances contraction of the heart adds to the mean resistance of the coronary vessels. In controlled studies of the effects of vagal stimulation on coronary blood flow, Heidenreich & Schmidt (382, 383) demonstrated that when the perfusion pressure is maintained at a constant level in the coronary arteries, vagal stimulation increases the blood flow, though under normal circumstances flow decreases because of simultaneous fall in aortic pressure. A similar study by Schreiner and co-workers (384) showed that, when the heart rate is maintained at a constant level by means of an electric pacemaker, the coronary blood flow increases following acetylcholine infusion. To summarize these different studies, it seems that the direct action of acetylcholine or vagal stimulation is to cause vasodilatation of the coronaries, though side effects on other parameters of heart function are likely to nullify this vasodilatation. Bartels and co-workers (385) have studied the difference in the degree of aeration of left atrial blood and femoral arterial blood, demonstrating in this way that as much as 16 per cent of the coronary blood flow returns to the heart by way of the thebesian vessels directly into

the left ventricle. This would explain about 30 to 50 per cent of the measured alveolar-femoral arterial oxygen pressure difference. Therefore, it is possible that the percentage of blood passing through right to left shunts in the lungs is somewhat less than previously believed.

Miscellaneous regional blood flow.—Hensel & Bender (386) have described a new heat conductivity apparatus for continuous determination of skin blood flow, and Davis & Hertzman (387) have described an improved photoelectric plethysmograph for analyzing vascular reactions in the nasal mucosa. By means of plethysmographic studies (388), studies on blood oxygen saturation from deep and superficial veins (389), and measurements of skin and hypothalamus temperature changes (390), it has been shown that body heating causes an increase in blood flow to the skin but essentially no change in muscle blood flow. Also, Green and co-workers (391) in studying the blood flow to the hindpaw of the dog showed that ischemia does not induce hyperemia but that both epinephrine and arterenol exert potent vasoconstrictor effects. No significant vasodilator effects transmitted by way of autonomic nerve fibers could be demonstrated. Thus, it seems that this vascular bed is controlled almost entirely by a potent sympathetic constrictor system.

In a particularly interesting study by Coles, Kidd & Patterson on the reactions of blood vessels in the human calf (392), it was demonstrated that negative pressures outside the leg at values greater than 50 mm. Hg cause an immediate vasoconstrictor effect, and that this is independent of interruption of the nerves. Though the cause of this effect is unknown, it could explain the automatic increase in vascular resistance in the legs when a person stands.

In studies on the renal circulation, Lilienfield and co-workers (393) and Ochwaldt (394) have all confirmed the fact that the transit time of red blood cells through the kidneys is considerably shorter than the transit time for plasma, and they showed respectively that the intrarenal hematocrit averages 89 and 90 per cent of the large vessel hematocrit. Nedeljkovic (395) has shown that renal blood flows estimated by means of para-amino hippurate correlate with direct measurements of blood flow only when the PAH concentration in the blood is stable for prolonged periods of time, and he recommends that indirect measurement of renal blood flow be discarded except when experimental conditions are extremely stable. Langston & Guyton (396) have shown that epinephrine and norepinephrine affect urine formation in two ways: first, by increasing the arterial pressure, which can directly increase urinary output, and, second, by acting directly on the vessels of the kidney to decrease the urinary output. Waugh & Hamilton (397), using kidneys perfused with oil, showed that a simultaneous rise in both venous and arterial pressure tends to dilate the renal vessels but also tends to increase intrarenal pressure which opposes vascular dilatation. Therefore, the two effects nullify each other, and the renal resistance remains constant despite the increased filling pressures in the vessels.

Reynolds (398) has reviewed the circulatory adaptations at birth, and in

guinea pigs he has demonstrated that immediately after birth the arterial capillaries of the lungs become extended during the process of pulmonary aeration, permitting the opening up of a previously closed capillary plexus (399). As a result of this, the pulmonary resistance greatly diminishes and pulmonary arterial pressure falls. Handler (400) has shown essentially the same physiological results in dogs, cats, and rabbits delivered by Caesarean section. Immediately after birth the pulmonary arterial pressure falls slightly below that in the descending aorta so that blood then flows mainly in a backward direction through the ductus. In newborn human beings, a few hours to a few days old, Adams & Lind (401) have also demonstrated a relatively large flow of blood from the aorta into the pulmonary artery, though at this time the pulmonary arterial pressure is still considerably elevated. To summarize, it appears that lung expansion at birth decreases the pulmonary resistance; this decrease in turn diminishes the pulmonary arterial pressure, allowing blood to flow from the aorta into the pulmonary artery. By means not yet determined, the backflow of blood gradually results in closure of the ductus.

The rate of blood flow through the thyroid gland of rabbits studied by I^{131} clearance and arteriovenous difference, utilizing the Fick principle (402), has been found to be 6.1 to 22 gm. per min. per gm. of thyroid. Using an electromagnetic flow meter, Cobbold & Lewis (403) have shown that blood flow to the knee joint is diminished by epinephrine and norepinephrine but increased by acetylcholine. Thus, at least in these respects, the vascular reactions of the joints appear to be similar to those of the skin.

LITERATURE CITED

1. Peterson, L. H., *Ann. Rev. Physiol.*, **19**, 255 (1957)
2. Bovet, D., and Carpi, A., *Ann. Rev. Physiol.*, **20**, 305 (1958)
3. Mertz, D., *Klin. Wochschr.*, **34**, 887 (1956)
4. Wasserman, K., Joseph, J. D., and Mayerson, H. S., *Am. J. Physiol.*, **184**, 175 (1956)
5. Shirley, H. H., Jr., Wolfram, C. G., Wasserman, K., and Mayerson, H. S., *Am. J. Physiol.*, **190**, 189 (1957)
6. Friedman, J. J., *Am. J. Physiol.*, **191**, 115 (1957)
7. Huggins, R. A., Smith, E. L., and Seibert, R. A., *Am. J. Physiol.*, **816**, 92 (1956)
8. Smith, E. L., Huggins, R. A., Krantz, L., Seibert, R. A., and Deavers, S., *Am. J. Physiol.*, **186**, 97 (1956)
9. Huggins, R. A., Smith, E. L., Deavers, S., and Overton, R. C., *Am. J. Physiol.*, **189**, 249 (1957)
10. Deavers, S., Huggins, R. A., and Smith, E. L., *Am. J. Physiol.*, **191**, 159 (1957)
11. Huggins, R. A., Smith, E. L., and Deavers, S., *Am. J. Physiol.*, **191**, 163 (1957)
12. Vidt, D. G., and Sapirstein, L. A., *Circulation Research*, **5**, 129 (1957)
13. Sjostrand, T., *Klin. Wochschr.*, **34**, 561 (1956)
14. Kaufmann, W., Klusmann, F. W., Koch, J., and Lutcke, A., *Arch. ges. Physiol.*, **263**, 253 (1956)
15. Henry, J. P., Gauer, O. H., and Reeves, J. L., *Circulation Research*, **4**, 85 (1956)

16. Zuidema, G. D., Clarke, N. P., Reeves, J. L., Gauer, O. H., and Henry, J. P., *Am. J. Physiol.*, **186**, 89 (1956)
17. Gauer, O. H., and Henry, J. P., *Klin. Wochschr.*, **34**, 356 (1956)
18. Gauer, O. H., Henry, J. P., and Sieker, H. O., *Circulation Research*, **4**, 79 (1956)
19. Finnerty, F. A., Jr., Buchholz, J. H., and Guillaudeu, R. L., *J. Clin. Invest.*, **37**, 425 (1958)
20. Rose, J. C., and Freis, E. D., *Am. J. Physiol.*, **191**, 283 (1957)
21. Fine, D., Meiselas, L. E., and Auerbach, T., *J. Clin. Invest.*, **37**, 232 (1958)
22. Bartter, F. C., Liddle, G. W., Duncan, L. E., Jr., Barber, J. K., and Delea, C., *J. Clin. Invest.*, **35**, 1306 (1956)
23. Coles, D. R., Kidd, B. S., and Moffat, W., *J. Appl. Physiol.*, **10**, 461 (1957)
24. Coles, D. R., and Patterson, G. C., *J. Physiol. (London)*, **135**, 163 (1957)
25. Lanari, A., *Circulation Research*, **5**, 236 (1957)
26. Eckstein, J. W., and Hamilton, W. K., *J. Clin. Invest.*, **36**, 1663 (1957)
27. Zweifach, B. W., *Am. J. Med.*, **23**, 684 (1957)
28. Illig, L., *Klin. Wochschr.*, **35**, 7 (1957)
29. Tischendorf, F., and Curri, S. B., *Z. mikroskop.-anat. Forsch.*, **62**, 326 (1956)
30. Hurley, H. J., Jr., and Mescon, H., *J. Appl. Physiol.*, **9**, 82 (1956)
31. Hyman, C., *Angéiologie*, **9**, 25 (1957)
32. Piiper, J., and Schürmeyer, E., *Arch. ges. Physiol.*, **263**, 364 (1956)
33. Piiper, J., *Angéiologie*, **9**, 19 (1957)
34. Lambert, J., *Angéiologie*, **9**, 11 (1957)
35. Kramar, J., Levine, V. E., Meyers, V. W., and Sass, R. N., *Am. J. Physiol.*, **192**, 603 (1958)
36. Gabor, M., and Dux, E., *Endokrinologie*, **34**, 225 (1957)
37. Menkin, V., *Am. J. Physiol.*, **189**, 98 (1957)
38. Paskhina, T. S., *Clin. Chim. Acta*, **1**, 24 (1956)
39. Heite, H. J., and Schrader, C. P., *Klin. Wochschr.*, **35**, 292 (1957)
40. Schega, H. W., *Deut. med. Wochschr.*, **82**, 470 (1957)
41. Barlow, G., Agersborg, H. P., Jr., and Overman, R. R., *Circulation Research*, **5**, 419 (1957)
42. Ott, H., *Klin. Wochschr.*, **34**, 1079 (1956)
43. Agostoni, E., Taglietti, A., and Setnikar, I., *Am. J. Physiol.*, **191**, 277 (1957)
44. Paulet, G., and Andre, P., *Arch. intern. physiol. et biochem.*, **65**, 401 (1957)
45. Frick, E., and Scheid-Seydel, L., *Klin. Wochschr.*, **36**, 66 (1958)
46. Mulrow, P. J., Oestreich, H. M., and Swan, R. C., *Am. J. Physiol.*, **185**, 179 (1956)
47. Ikkos, D., *Acta Physiol. Scand.*, **35**, 240 (1956)
48. Ikkos, D., Ljunggren, H., Luft, R., and Sjögren, B., *Acta Physiol. Scand.*, **35**, 254 (1956)
49. Harrison, C. S., and Faler, K. T., *Am. J. Physiol.*, **188**, 568 (1957)
50. White, H. L., and Rolf, D., *J. Clin. Invest.*, **37**, 8 (1958)
51. Bresler, E. H., *Am. J. Med. Sci.*, **232**, 93 (1956)
52. Smith, H. W., *Am. J. Med.*, **23**, 623 (1957)
53. Reinhardt, W. O., and Yoffey, J. M., *Am. J. Physiol.*, **187**, 493 (1956)
54. Hughes, R., May, A. J., and Widdicombe, J. G., *J. Physiol. (London)*, **132**, 384 (1956)
55. Benson, J. A., Jr., Lee, P. R., Scholer, J. F., Kim, K. S., and Bollman, J. L., *Am. J. Physiol.*, **184**, 441 (1956)

56. Goodwin, W. E., and Kaufman, J. J., *Surg. Forum, Proc. 40th Cong. Am. Coll. Surgeons*, **6**, 632 (1956)
57. Goodwin, W. E., and Kaufman, J. J., *J. Urol.*, **76**, 702 (1956)
58. Papamiltiades, M., *Ann. oculist (Paris)*, **189**, 939 (1956)
59. Langham, M. E., *Physiol. Revs.*, **38**, 215 (1958)
60. Lammerant, J., Sprumont, P., and de Visscher, M., *Arch. intern. physiol. et biochem.*, **64**, 65 (1956)
61. Emanuel, R. W., Lacy, W. W., and Newman, E. V., *Circulation Research*, **5**, 527 (1957)
62. Lacy, W. W., Emanuel, R. W., and Newman, E. V., *Circulation Research*, **5**, 568 (1957)
63. Goodwin, R. S., and Sapirstein, L. A., *Circulation Research*, **5**, 531 (1957)
64. Lochner, W., and dal Ri, H., *Arch. ges. Physiol.*, **264**, 453 (1957)
65. Cleempoel, H., and Bertinchamps, A., *Acta cardiol.*, **12**, 675 (1957)
66. Huff, R. L., Parrish, D., and Crockett, W., *Circulation Research*, **5**, 395 (1957)
67. Fritts, H. W., Jr., Harris, P., Chidsey, C. A., III, Clauss, R. H., and Cournand, A., *J. Appl. Physiol.*, **11**, 362 (1957)
68. Shumway, N. E., Johnson, J. A., and Stish, R. J., *J. Appl. Physiol.*, **10**, 297 (1957)
69. Ring, G. C., Moss, W. G., Reiner, I., Partin, H., and Kurbatov, T., *Am. J. Physiol.*, **187**, 283 (1956)
70. Keys, J. R., Hetzel, P. S., and Wood, E. H., *J. Appl. Physiol.*, **11**, 385 (1957)
71. Kramer, K., and Ziegenrucker, G., *Klin. Wochschr.*, **35**, 468 (1957)
72. Gerst, P., Taylor, C., and Peterson, L. H., *Am. J. Physiol.*, **189**, 191 (1957)
73. Brotmacher, L., *Circulation Research*, **5**, 589 (1957)
74. Guyton, A. C., Nichols, R. J., Jr., and Farish, C., *J. Appl. Physiol.*, **10**, 158 (1957)
75. Scharf, R., *Acta cardiol.*, **12**, 493 (1957)
76. Fleming, J. W., and Bloom, W. L., *J. Clin. Invest.*, **36**, 1233 (1957)
77. Gauer, O. H., *Verhandl. deut. Ges. Kreislaufforsch.*, **22**, 61 (1956)
78. Rose, J. C., Lazaro, E. J., and Broida, H. P., *Circulation Research*, **4**, 173 (1956)
79. Freis, E. D., and Rose, J. C., *Am. J. Med.*, **22**, 175 (1957)
80. Guyton, A. C., Lindsey, A. W., Abernathy, B., and Langston, J. B., *Am. J. Physiol.*, **192**, 126 (1958)
81. Reichel, H., *Verhandl. deut. Ges. Kreislaufforsch.*, **22**, 3 (1956)
82. Rose, J. C., and Freis, E. D., *Am. J. Physiol.*, **191**, 283 (1957)
83. Leusen, I., Demeester, G., and Bouckaert, J. J., *Arch. intern. physiol. et biochem.*, **64**, 489 (1956)
84. Weissler, A. M., Leonard, J. J., and Warren, J. V., *J. Clin. Invest.*, **36**, 1656 (1957)
85. Combes, B., Preedy, J. R. K., Wheeler, H. O., Hays, R. M., and Bradley, S. E., *J. Clin. Invest.*, **36**, 860 (1957)
86. Trapold, J. H., *Circulation Research*, **5**, 444 (1957)
87. Weissler, A. M., Warren, J. V., Estes, E. H., Jr., McIntosh, H. D., and Leonard, J. J., *Circulation*, **15**, 875 (1957)
88. Blumberger, K., *Verhandl. deut. Ges. Kreislaufforsch.*, **22**, 79 (1956)
89. Fowler, N. O., Franch, R. H., and Bloom, W. L., *Circulation Research*, **4**, 319 (1956)

90. Fowler, N. O., Bloom, W. L., and Ward, J. A., *Circulation Research*, **6**, 163 (1958)
91. Leusen, I., Demeester, G., and Bouckaert, J. J., *Arch. intern. physiol. et biochem.*, **64**, 564 (1956)
92. Wégria, R., Nakano, J., McGiff, J. C., Rochester, D. F., Blumenthal, M. R., and Muraviev, T., *Am. J. Physiol.*, **193**, 147 (1958)
93. Rodbard, S., Krause, S., Lowenthal, M., and Katz, L. N., *Am. J. Physiol.*, **187**, 458 (1956)
94. Smith, V. M., Hughes, C. W., Sapp, O., Joy, R. J. T., and Mattingly, T. W., *Arch. Internal Med.*, **100**, 833 (1957)
95. Asmussen, E., and Nielsen, M., *Acta Physiol. Scand.*, **35**, 73 (1956)
96. Guyton, A. C., *World Trends in Cardiology*, **III**, 32 (Paul B. Hoeber, Inc., New York, N. Y., 131 pp., 1956)
97. Guyton, A. C., Lindsey, A. W., Abernathy, B., and Richardson, T., *Am. J. Physiol.*, **189**, 609 (1957)
98. Fejar, E., *Acta cardiol.*, **12**, 13 (1957)
99. Kaufman, W. C., and Marbarger, J. P., *J. Appl. Physiol.*, **9**, 33 (1956)
100. Braunwald, E., Binion, J. T., Morgan, W. L., Jr., and Sarnoff, S. J., *Circulation Research*, **5**, 670 (1957)
101. Morgan, W. L., Jr., Binion, J. T., and Sarnoff, S. J., *J. Appl. Physiol.*, **10**, 26 (1957)
102. Binion, J. T., Morgan, W. L., Jr., Welch, H. G., and Sarnoff, S. J., *Circulation Research*, **4**, 705 (1956)
103. Gauer, O. H., and Sieker, H. O., *Circulation Research*, **4**, 74 (1956)
104. Duomarco, J. L., Rimini, R., and Giambruno, C. E., *Acta Physiol. Latinoam.*, **7**, 8 (1957)
105. Freis, E. D., Schnaper, H. W., and Lilienfeld, L. S., *J. Clin. Invest.*, **36**, 245 (1957)
106. Sapirostein, L. A., *Am. J. Physiol.*, **193**, 161 (1958)
107. Dobson, E. L., and Warner, G. F., *Am. J. Physiol.*, **189**, 269 (1957)
108. Sapirostein, L. A., and Ogden, E., *Circulation Research*, **4**, 245 (1956)
109. Allwood, M. J., *Circulation Research*, **4**, 268 (1956)
110. Clarke, R. S. J., Ginsburg, J., and Hellon, R. F., *J. Physiol. (London)*, **140**, 318 (1958)
111. Imig, C. J., Caskill, H., Jr., Bauer, A., and Hines, H. M., *Arch. Phys. Med. Rehabil.*, **38**, 571 (1957)
112. Winsor, T., *Angiology*, **8**, 87 (1957)
113. Wever, R., and Aschoff, J., *Arch. ges. Physiol.*, **264**, 272 (1957)
114. Aschoff, J., Heinemann, W., and Wever, R., *Arch. ges. Physiol.*, **264**, 191 (1957)
115. Hierholzer, K., Frehner, K., and Schleier, S., *Arch. ges. Physiol.*, **264**, 94 (1957)
116. Hilger, H., and Brechtelsbauer, H., *Arch. ges. Physiol.*, **263**, 615 (1956)
117. Janssen, S., *Arch. ges. Physiol.*, **624**, 198 (1957)
118. Fry, D. L., Mallos, A. J., and Casper, A. G. T., *Circulation Research*, **4**, 627 (1956)
119. Fry, D. L., Noble, F. W., and Mallos, A. J., *Circulation Research*, **5**, 75 (1957)
120. Williams, M. H., Jr., *J. Appl. Physiol.*, **9**, 299 (1956)
121. Wretling, A., *Acta Physiol. Scand.*, **40**, 196 (1957)
122. Levy, M. N., *Circulation Research*, **4**, 533 (1956)
123. Levy, M. N., *Am. J. Physiol.*, **192**, 164 (1958)

124. Guyton, A. C., Lindsey, A. W., and Armstrong, G. G., *Am. J. Physiol.*, **186**, 294 (1956)
125. Read, R. C., Kuida, H., and Johnson, J. A., *Am. J. Physiol.*, **192**, 609 (1958)
126. Folkow, B., and Lofving, B., *Acta Physiol. Scand.*, **38**, 37 (1956)
127. Roddie, I. C., and Shepherd, J. T., *J. Physiol. (London)*, **136**, 498 (1957)
128. Read, R. C., Kuida, H., and Johnson, J. A., *Circulation Research*, **5**, 676 (1957)
129. Haddy, F. J., *Circulation Research*, **4**, 659 (1956)
130. Haddy, F. J., Fleishman, M., and Scott, J. B., *Circulation Research*, **5**, 58 (1957)
131. Haddy, F. J., Fleishman, M., and Emanuel, D. A., *Circulation Research*, **5**, 247 (1957)
132. Fleishman, M., Scott, J., and Haddy, F. J., *Circulation Research*, **5**, 602 (1957)
133. Haddy, F. J., and Gilbert, R. P., *Circulation Research*, **4**, 25 (1956)
134. Kelly, W. D., and Visscher, M. B., *Am. J. Physiol.*, **185**, 453 (1956)
135. Maude, A. D., and Whitmore, R. L., *J. Appl. Physiol.*, **12**, 105 (1958)
136. Lanari, A., Bromberger-Barnea, B., and Attinger, E., *J. Appl. Physiol.*, **9**, 69 (1956)
137. Comens, P., and Schroeder, H. A., *J. Appl. Physiol.*, **11**, 181 (1957)
138. Golenhofen, K., and Hilderbrandt, G., *Arch. ges. Physiol.*, **264**, 492 (1957)
139. Patel, D. J., and Burton, A. C., *Circulation Research*, **4**, 710 (1956)
140. Barcroft, H., and Cobbold, A. F., *J. Physiol. (London)*, **132**, 372 (1956)
141. Muller, E. A., *Arch. ges. Physiol.*, **265**, 429 (1958)
142. Lausen, I., Demeester, G., and Bouckaert, J. J., *Arch. intern. physiol. et biochem.*, **64**, 564 (1956)
143. Lambert, J., *Arch. intern. physiol. et biochem.*, **64**, 623 (1956)
144. Lambert, J., *Arch. intern. physiol. et biochem.*, **65**, 46 (1957)
145. Lambert, J., *Arch. intern. physiol. et biochem.*, **65**, 69 (1957)
146. Berglund, E., Monroe, R. G., and Schreiner, G. L., *Acta Physiol. Scand.*, **41**, 261 (1957)
147. Laurent, D., Bolene-Williams, C., Williams, F. L., and Katz, L. N., *Am. J. Physiol.*, **185**, 355 (1956)
148. Michel, D., Nocker, J., and Hartleb, O., *Klin. Wochschr.*, **34**, 701 (1956)
149. McNeill, T. A., *J. Physiol. (London)*, **134**, 195 (1956)
150. Thorban, W., and Schonbach, G., *Bull. schweiz. Akad. med. Wiss.*, **13**, 458 (1957)
151. Rudolph, A. M., and Paul, M. H., *J. Appl. Physiol.*, **10**, 327 (1957)
152. Lee, J. S., *J. Appl. Physiol.*, **10**, 329 (1957)
153. Zuidema, G. D., and Edelberg, R., *J. Appl. Physiol.*, **9**, 501 (1956)
154. Knudsen, P. J., *Acta Physiol. Scand.*, **39**, 137 (1957)
155. Stegemann, J., *Arch. ges. Physiol.*, **262**, 419 (1956)
156. Starr, I., and Schild, A., *J. Appl. Physiol.*, **11**, 169 (1957)
157. Starr, I., *J. Appl. Physiol.*, **11**, 174 (1957)
158. Eger, W., and Klensch, H., *Arch. ges. Physiol.*, **262**, 443 (1956)
159. Klensch, H., and Eger, W., *Arch. ges. Physiol.*, **263**, 459 (1956)
160. Klensch, H., Hohnen, H. W., and Kessler, K. H., *Arch. ges. Physiol.*, **264**, 424 (1957)
161. Klensch, H., and Hohnen, H. W., *Arch. ges. Physiol.*, **265**, 207 (1957)
162. Hohnen, H. W., and Klensch, H., *Arch. ges. Physiol.*, **265**, 199 (1957)
163. Oberholzer, R. J. H., *Klin. Wochschr.*, **35**, 448 (1957)
164. Lindgren, P., *Acta Physiol. Scand.*, **35**, 121 (1956)

165. Morin, G., Corriol, J., and Zwirn, P., *Compt. rend. soc. biol.*, **150**, 750 (1956)
166. Roddie, I. C., Shepherd, J. T., and Whelan, R. F., *J. Physiol. (London)*, **136**, 489 (1957)
167. Langford, H. G., Patterson, J. L., and Porter, R. R., *Circulation Research*, **5**, 268 (1957)
168. Fronkova, K., Ehrlich, V., and Sleg, L., *Arch. ges. Physiol.*, **263**, 704 (1956)
169. Bock, K. D., and Bonhoeffer, K., *Arch. ges. Physiol.*, **263**, 93 (1956)
170. Tenney, S. M., *Am. J. Physiol.*, **187**, 341 (1956)
171. Neil, E., *Arch. intern. pharmacodynamie*, **105**, 468 (1956)
172. Brind, S. H., Bianchine, J. R., and Levy, M. N., *Am. J. Physiol.*, **185**, 483 (1956)
173. Kalkoff, W., *Arch. ges. Physiol.*, **265**, 81 (1957)
174. Overbeck, W., and Koepchen, H. P., *Arch. ges. Physiol.*, **263**, 553 (1956)
175. Lawton, R. W., Greene, L. C., Kydd, G. H., Peterson, L. H., and Crosbie, R. J., *J. Aviation Med.*, **29**, 97 (1958)
176. Warner, H. R., *Circulation Research*, **6**, 35 (1958)
177. Hilton, J. G., and Brown, R. V., *Am. J. Physiol.*, **186**, 71 (1956)
178. Carlsten, A., Folkow, B., and Hamberger, C. A., *Acta Physiol. Scand.*, **41**, 68 (1957)
179. Boss, J., and Green, J. H., *Circulation Research*, **4**, 12 (1956)
180. Douglas, W. W., Ritchie, J. M., and Schaumann, W., *J. Physiol. (London)*, **133**, 232 (1956)
181. Wagner, R., *Circulation Research*, **5**, 469 (1957)
182. Brown, F. K., *Am. J. Physiol.*, **185**, 510 (1956)
183. Mukherjee, S. R., *J. Physiol. (London)*, **138**, 307 (1957)
184. Mukherjee, S. R., *J. Physiol. (London)*, **138**, 300 (1957)
185. Rohse, W. G., Kaye, M., and Randall, W. C., *Circulation Research*, **5**, 144 (1957)
186. Folkow, B., and Hamberger, C. A., *J. Appl. Physiol.*, **9**, 268 (1956)
187. Douglas, W. W., and Ritchie, J. M., *J. Physiol. (London)*, **134**, 167 (1956)
188. Capon, A., *Arch. intern. physiol. et biochem.*, **65**, 515 (1957)
189. DiSalvo, R. J., Bloom, W. L., Brust, A. A., Ferguson, R. W., and Ferris, E. B., *J. Clin. Invest.*, **35**, 568 (1956)
190. Lofving, B., and Mellander, S., *Acta Physiol. Scand.*, **37**, 134 (1956)
191. Burch, G. E., and Murtadha, M., *Am. Heart J.*, **51**, 807 (1956)
192. Alexander, R. W., *Circulation Research*, **4**, 49 (1956)
193. Salzman, E. W., and Leverett, S. D., Jr., *Circulation Research*, **4**, 540 (1956)
194. Salzman, E. W., *Circulation Research*, **5**, 149 (1957)
195. Lee, J. S., and Visscher, M. B., *Am. J. Physiol.*, **190**, 37 (1957)
196. Mooring, P. K., Rathe, J., and Root, W. S., *Am. J. Physiol.*, **186**, 85 (1956)
197. Hilton, J. G., and Reid, S. C., *Am. J. Physiol.*, **186**, 289 (1956)
198. Hille, H., and Teske, H.-J., *Arch. ges. Physiol.*, **263**, 83 (1956)
199. Kärki, N. T., *Acta Physiol. Scand.*, **39**, Suppl. 132, 1 (1957)
200. Pitkanen, E., *Acta Physiol. Scand.*, **38**, Suppl. 129, 1 (1956)
201. Goodall, McC., and Kirshner, N., *Circulation*, **17**, 366 (1958)
202. von Euler, U. S., and Franksson, C., *Acta Physiol. Scand.*, **38**, 275 (1956)
203. Duner, H., and von Euler, U. S., *Acta Physiol. Scand.*, **38**, 355 (1956)
204. Lundholm, L., *Acta Physiol. Scand.*, **39**, Suppl. 133, 1 (1957)
205. Lundholm, L., *Acta Physiol. Scand.*, **40**, 344 (1957)

206. Deal, C. P., Jr., and Green, H. D., *Circulation Research*, **4**, 38 (1956)
207. Ottis, K., Davis, J. E., Jr., and Green, H. D., *Am. J. Physiol.*, **189**, 599 (1957)
208. Brose, W., and Oberdorf, A., *Arch. ges. Physiol.*, **263**, 68 (1956)
209. Deal, C. P., and Green, H. D., *Circulation Research*, **4**, 38 (1956)
210. Denison, A. B., Jr., Bardhanabaedya, S., and Green, H. D., *Circulation Research*, **4**, 653 (1956)
211. Glover, W. E., Greenfield, A. D. M., Kidd, B. S., and Whelan, R. F., *J. Physiol. (London)*, **140**, 113 (1958)
212. Farrand, E., Allbaugh, L. R., and Horvath, S. M., *Am. J. Physiol.*, **189**, 576 (1957)
213. Richardson, J. A., Woods, E. F., and Richardson, A. K., *J. Pharmacol. Exptl. Therap.*, **119**, 378 (1957)
214. Grette, K., *Acta Pharmacol. Toxicol.*, **13**, 177 (1957)
215. Rosell, S., Uvnas, B., and Wretling, A., *Acta Pharmacol. Toxicol.*, **13**, 289 (1957)
216. Davidson, J., Sjoerdsma, A., Loomis, L. N., and Udenfriend, S., *J. Clin. Invest.*, **36**, 1594 (1957)
217. Rudolph, A. M., and Paul, M. H., *Am. J. Physiol.*, **189**, 263 (1957)
218. Spinazzola, A. J., and Sherrod, T. R., *J. Pharmacol. Exptl. Therap.*, **119**, 114 (1957)
219. Blatteis, C. M., and Horvath, S. M., *Am. J. Physiol.*, **192**, 353 (1958)
220. Nash, C. B., Davis, F., and Woodbury, R. A., *Am. J. Physiol.*, **185**, 107 (1958)
221. Lindell, S. E., *Acta Physiol. Scand.*, **41**, 168 (1957)
222. Rosen, A., Strandberg, P., and Uvnas, B., *Acta Pharmacol. Toxicol.*, **13**, 267 (1957)
223. Lindell, S. E., and Westling, H., *Acta Physiol. Scand.*, **37**, 307 (1956)
224. Fakstorp, J., Pedersen, J. G. A., Poulsen, E., and Schilling, M., *Acta Pharmacol. Toxicol.*, **13**, 52 (1956)
225. Fakstorp, J., and Pedersen, J. G. A., *Acta Pharmacol. Toxicol.*, **13**, 359 (1957)
226. Frederiksson, T., *Acta Pharmacol. Toxicol.*, **13**, 86 (1956)
227. Maxwell, R. A., Plummer, A. J., and Osborne, M. W., *Circulation Research*, **4**, 276 (1956)
228. Rose, J. C., and Lazaro, E. J., *J. Pharmacol. Exptl. Therap.*, **117**, 461 (1956)
229. Sturtevant, F. M., *J. Pharmacol. Exptl. Therap.*, **121**, 369 (1957)
230. Page, I. H., *Physiol. Revs.*, **38**, 277 (1958)
231. Lewis, G. P., *5-Hydroxytryptamine* (Pergamon Press, Inc., New York, N. Y., 253 pp., 1958)
232. Faulconer, A., Jr., and Patrick, R. T., *Ann. Rev. Med.*, **8**, 349 (1957)
233. Remington, J. W., and Wood, E. H., *J. Appl. Physiol.*, **9**, 433 (1956)
234. Landowne, M., *J. Appl. Physiol.*, **12**, 91 (1958)
235. Landowne, M., *Circulation Research*, **5**, 594 (1957)
236. Warner, H. R., *Circulation Research*, **5**, 79 (1957)
237. Randall, J. E., and Stacy, R. W., *Am. J. Physiol.*, **187**, 94 (1956)
238. Randall, J. E., and Stacy, R. W., *Am. J. Physiol.*, **185**, 351 (1956)
239. Ryan, J. M., Stacy, R. W., and Watman, R. N., *Circulation Research*, **4**, 676 (1956)
240. Roach, M. R., and Burton, A. C., *Can. J. Biochem. and Physiol.*, **35**, 681 (1957)
241. Wetterer, E., *Verhandl. deut. Ges. Kreislaufforsch.*, **22**, 26 (1956)
242. Spencer, M. P., and Denison, A. B., *Circulation Research*, **4**, 476 (1956)
243. Schroeder, W., and Freund, H., *Arch. ges. Physiol.*, **262**, 456 (1956)

244. Davey, D. A., *J. Physiol. (London)*, **132**, 1 (1956)
245. Lippay, F., *Australian J. Exptl. Biol. Med. Sci.*, **34**, 301 (1956)
246. Leonard, E., and Sarnoff, S. J., *Circulation Research*, **5**, 169 (1957)
247. Muirhead, E. E., Jones, F., and Stirman, J. A., *Am. J. Physiol.*, **191**, 537 (1957)
248. Grollman, A., and White, F. N., *Am. J. Physiol.*, **193**, 144 (1958)
249. Crandall, E. E., McCrorey, H. L., Sukowski, E. J., and Wakerlin, G. E., *Circulation Research*, **5**, 683 (1957)
250. Culbertson, J. W., Eckstein, J. W., Kirkendall, W. M., and Bedell, G. N., *J. Clin. Invest.*, **36**, 1537 (1957)
251. Bock, K. D., Krecke, H. J., and Kuhn, H. M., *Klin. Wochschr.*, **36**, 254 (1958)
252. Skeggs, L. T., Jr., Kahn, J. R., and Shunway, N. P., *J. Exptl. Med.*, **103**, 295 (1956)
253. Helmer, O. M., *Am. J. Physiol.*, **188**, 571 (1957)
254. Skeggs, L. T., Jr., Kahn, J. R., and Shumway, N. P., *J. Exptl. Med.*, **103**, 301 (1956)
255. Schapiro, S., Gordon, D. B., and Drury, D. R., *Am. J. Physiol.*, **185**, 543 (1956)
256. Rothman, S., and Drury, D. R., *Am. J. Physiol.*, **188**, 371 (1957)
257. Vanatta, J. C., Danhof, I., and McMullen, M., *Am. J. Physiol.*, **185**, 167 (1956)
258. Sturtevant, F. M., *Am. Heart J.*, **52**, 410 (1956)
259. Mendlowitz, M., Torosdag, S. M., and Sharney, L., *J. Appl. Physiol.*, **10**, 436 (1957)
260. Redleaf, P. D., and Tobian, L., *Circulation Research*, **6**, 185 (1958)
261. Skelton, F. R., *Lab. Invest.*, **6**, 266 (1957)
262. Sarre, H., Kampmann, W., and Schmidt, G., *Klin. Wochschr.*, **34**, 509 (1956)
263. Hollander, W., and Judson, W. E., *J. Clin. Invest.*, **36**, 1460 (1957)
264. Tobian, L., *Circulation Research*, **4**, 671 (1956)
265. Katz, J. I., Skom, J. H., and Wakerlin, G. E., *Circulation Research*, **5**, 137 (1957)
266. Baillet, J., *Compt. rend.*, **243**, 91 (1956)
267. Fregly, M. J., and Cook, C. S., *Am. J. Physiol.*, **187**, 293 (1956)
268. Fregly, M. J., *Am. J. Physiol.*, **191**, 542 (1957)
269. Fregly, M. J., *Am. J. Physiol.*, **187**, 288 (1956)
270. Capon, A., *Arch. intern. physiol. et biochem.*, **66**, 105 (1958)
271. Smith, J. R., and Hoobler, S. W., *Circulation*, **14**, 1061 (1956)
272. Milnor, W. R., and Crary, H. I., *J. Appl. Physiol.*, **11**, 97 (1957)
273. Samet, P., Fritts, H. W., Jr., Fishman, A. P., and Courmand, A., *Medicine*, **36**, 211 (1957)
274. Remenchik, A. P., and Moorhouse, J. A., *Arch. Internal Med.*, **100**, 445 (1957)
275. Decortis, A., *Acta cardiol.*, **12**, 240 (1957)
276. Davis, J. O., Goodkind, M. J., Pechet, M. M., and Ball, W. C., Jr., *Am. J. Physiol.*, **187**, 45 (1956)
277. Davis, J. O., Pechet, M. M., Ball, W. C., Jr., and Goodkind, M. J., *J. Clin. Invest.*, **36**, 689 (1957)
278. Ball, W. C., Jr., Davis, J. O., and Goodkind, M. J., *Am. J. Physiol.*, **188**, 578 (1957)
279. Davis, J. O., Bahn, R. C., Goodkind, M. J., and Ball, W. C., Jr., *Am. J. Physiol.*, **191**, 329 (1957)
280. Ball, W. C., Jr., and Davis, J. O., *Am. J. Physiol.*, **191**, 339 (1957)
281. Davis, J. O., Goodkind, M. J., and Ball, W. C., Jr., *Circulation Research*, **5**, 388 (1957)

282. Davis, J. O., and Ball, W. C., Jr., *Am. J. Physiol.*, **192**, 538 (1958)
283. Goodkind, M. J., Ball, W. C., Jr., and Davis, J. O., *Am. J. Physiol.*, **189**, 181 (1957)
284. Driscoll, T. E., Maultsby, M. M., Farrell, G. L., and Berne, R. M., *Am. J. Physiol.*, **191**, 140 (1957)
285. Jacobson, J. H., II, Laragh, J., and McAllister, F. F., *Surg. Forum. Proc.*, **7**, 45 (1956)
286. Underwood, R. J., Griswold, H. E., and Hurst, W. W., *Circulation Research*, **5**, 257 (1957)
287. Alexander, N., Henshaw, L. B., and Drury, D. R., *Circulation Research*, **5**, 375 (1957)
288. Alexander, N., and Drury, D. R., *Am. J. Physiol.*, **191**, 476 (1957)
289. Foldi, M., Solti, F., Koltay, E., Megyesi, K., Rev, J., and Szasz, J., *Klin. Wochschr.*, **34**, 857 (1956)
290. Wood, J., Litter, J., and Wilkins, R. W., *Circulation*, **13**, 524 (1956)
291. Manger, W. M., Bollman, J. L., Maher, F. T., and Berkson, J., *Am. J. Physiol.*, **190**, 310 (1957)
292. Farrell, G. L., Rosnagle, R. S., and Rauschkolb, E. W., *Circulation Research*, **4**, 606 (1956)
293. Jacob, S., Friedman, E. W., Levenson, S., Glotzer, P., Frank, H. A., and Fine, J., *Am. J. Physiol.*, **186**, 79 (1956)
294. Crowell, J. W., Bounds, S. H., and Johnson, W. W., *Am. J. Physiol.*, **192**, 171 (1958)
295. Crowell, J. W., and Smith, E. E., *Am. J. Physiol.*, **186**, 283 (1956)
296. Hirsch, H., Euler, K. H., and Schneider, M., *Arch. ges. Physiol.*, **275**, 281 (1957)
297. Smith, J. J., and Grace, R. A., *Am. J. Physiol.*, **191**, 135 (1957)
298. Johnson, P. C., and Selkurt, E. E., *Am. J. Physiol.*, **193**, 135 (1958)
299. Selkurt, E. E., and Brecher, G. A., *Circulation Research*, **4**, 693 (1956)
300. Cull, T. E., Scibetta, M. P., and Selkurt, E. E., *Am. J. Physiol.*, **185**, 365 (1956)
301. Koppen, H., *Arch. ges. Physiol.*, **264**, 64 (1957)
302. Hild, R., Mechelke, K., and Nusser, E., *Arch. ges. Physiol.*, **263**, 401 (1956)
303. White, R. P., and Woodard, P. H., *Am. J. Physiol.*, **188**, 189 (1957)
304. Lecomte, J., and de Berg, L. V., *Arch. intern. physiol. et biochem.*, **65**, 36 (1957)
305. Lecomte, J., *Arch. intern. physiol. et biochem.*, **65**, 451 (1957)
306. Brown, T. G., Jr., *J. S. Carolina Med. Assoc.*, **52**, 363 (1956)
307. Fisher, B., Russ, D., and Fedor, E. J., *Am. J. Physiol.*, **188**, 473 (1957)
308. Rose, J. C., McDermott, T. F., Lilienfeld, L. S., Porfido, F. A., and Kelley, R. T., *Circulation*, **15**, 512 (1957)
309. Nedzel, A. J., and Brown, J., *J. Aviation Med.*, **27**, 236 (1956)
310. Lynch, H. F., and Adolph, E. F., *J. Appl. Physiol.*, **11**, 192 (1957)
311. Hirsch, H., Euler, K. H., and Schneider, M., *Arch. ges. Physiol.*, **265**, 314 (1957)
312. Brown, G. M., *Rev. can. biol.*, **16**, 279 (1957)
313. Covino, B. G., and Beavers, W. R., *Am. J. Physiol.*, **191**, 153 (1957)
314. Imig, C. J., Roberson, W. J., and Hines, H. M., *Am. J. Physiol.*, **186**, 35 (1956)
315. Carlill, S. D., and Duke, H. N., *J. Physiol. (London)*, **133**, 275 (1956)
316. Borst, H. G., McGregor, M., Whittenberger, J. L., and Berglund, E., *Circulation Research*, **4**, 393 (1956)
317. Piiper, J., *Arch. ges. Physiol.*, **264**, 596 (1957)
318. Burton, A. C., and Patel, D. J., *J. Appl. Physiol.*, **12**, 239 (1958)

319. Harrison, R. W., Adams, W. E., Beuhler, W., and Long, E. T., *Arch. Surg.*, **75**, 546 (1957)
320. Hanson, H. E., *Scand. J. Clin. & Lab. Invest.*, **7**, 61 (1955)
321. Nemir, P., Jr., Stone, H. H., Hawthorne, H. R., and Mackrell, T. N., *J. Thoracic Surg.*, **32**, 562 (1956)
322. Brofman, B. L., Charms, B. L., Kohn, P. M., Elder, J., Newman, R., and Rizeka, M., *J. Thoracic Surg.*, **34**, 206 (1957)
323. Leusen, I., Demeester, G., and Vuylsteek, K., *Acta cardiol.*, **12**, 1 (1957)
324. Vuylsteek, K., Van Loo, A., Leusen, I., Straeten, M. van der, Verstraeten, J., Rotgens, M., and Pannier, R., *Verhandl. deut. Ges. Kreislaufforsch.*, **22**, 229 (1956)
325. Lategola, M. T., *Am. J. Physiol.*, **192**, 613 (1958)
326. DuBois, A. B., and Marshall, R., *J. Clin. Invest.*, **36**, 1566 (1957)
327. Cournand, A. F., *Am. Heart J.*, **54**, 172 (1957)
328. Borst, H. G., Berglund, E., and McGregor, M., *J. Clin. Invest.*, **36**, 669 (1957)
329. Patel, D. J., and Burton, A. C., *Circulation Research*, **5**, 620 (1957)
330. Fritts, H. W., Jr., Harris, P., Clauss, R. H., Odell, J. E., and Cournand, A., *J. Clin. Invest.*, **37**, 99 (1958)
331. Rose, J. C., *Proc. Soc. Exptl. Biol. Med.*, **94**, 734 (1957)
332. Borst, H. G., Whittenberger, J. L., Berglund, E., and McGregor, M., *Am. J. Physiol.*, **191**, 446 (1957)
333. Aviado, D. M., Jr., Ling, J. S., and Schmidt, C. F., *Am. J. Physiol.*, **189**, 253 (1957)
334. Peters, R. M., *Am. J. Physiol.*, **191**, 399 (1957)
335. de Burgh Daly, M., and Schweitzer, A., *J. Physiol. (London)*, **131**, 220 (1956)
336. de Burgh Daly, I., and de Burgh Daly, M., *J. Physiol. (London)*, **137**, 427 (1957)
337. Agostoni, E., Chinnock, J. E., and de Burgh Daly, M., *J. Physiol. (London)*, **137**, 447 (1957)
338. de Burgh Daly, I., and de Burgh Daly, M., *J. Physiol. (London)*, **137**, 436 (1957)
339. Ferri, F., Rovati, V., Panesi, M., Romanelli, R., and Righini, E., *Acta cardiol.*, **12**, 269 (1957)
340. Courtoy, P., and Salonikides, N., *Acta cardiol.*, **11**, 52 (1956)
341. Visscher, M. B., Haddy, F. J., and Stephens, G., *Pharmacol. Revs.*, **8**, 389 (1956)
342. Aravanis, C., Libretti, A., Jona, E., Polli, J. F., Liu, C. K., and Luisada, A. A., *Am. J. Physiol.*, **189**, 132 (1957)
343. Polli, J. F., and Luisada, A. A., *Am. J. Physiol.*, **188**, 599 (1957)
344. Hawthorne, E. W., Brownlee, G. V., and Jason, R. S., *Am. J. Physiol.*, **185**, 474 (1956)
345. Hawthorne, E. W., Brownlee, G. V., and Spellman, M., *Am. J. Physiol.*, **185**, 479 (1956)
346. Borst, H. G., Berglund, E., Whittenberger, J. L., Mead, J., McGregor, M., and Collier, C., *J. Clin. Invest.*, **36**, 1708 (1957)
347. Bauman, A., Rothschild, M. A., Yalow, R. S., and Berson, S. A., *J. Appl. Physiol.*, **11**, 353 (1957)
348. Fishman, A. P., Becker, E. L., Fritts, H. W., Jr., and Heinemann, H. O., *Am. J. Physiol.*, **188**, 95 (1957)
349. Rapaport, E., Kuida, H., Haynes, F. W., and Dexter, L., *Am. J. Physiol.*, **185**, 127 (1956)

350. Lindsey, A. W., Banahan, B. F., Cannon, R. H., and Guyton, A. C., *Am. J. Physiol.*, **190**, 45 (1957)
351. Lammerant, J., *Le volume sanguin des poumons chez l'homme* (Editions Arscia, Brussels, Belgium, pp. 1-192, 1957)
352. Comet, S., and Lagerlof, H. O., *Acta Physiol. Scand.*, **36**, 337 (1956)
353. Comet, S., and Lagerlof, H., *Acta Physiol. Scand.*, **36**, 348 (1956)
354. Lechner, W., Bartels, H., Beer, R., Mochizuki, M., and Roderwald, G., *Arch. ges. Physiol.*, **264**, 294 (1957)
355. Sancetta, S. M., and Rakita, L., *J. Clin. Invest.*, **36**, 1138 (1957)
356. Meyer, W. W., and Schollmeyer, P., *Klin. Wochschr.*, **35**, 1070 (1957)
357. Guyton, A. C., Crowell, J. W., and Moore, J. W., *Am. J. Physiol.*, **187**, 395 (1956)
358. Carlyle, A., and Grayson, J., *J. Physiol. (London)*, **133**, 10 (1956)
359. Finnerty, F. A., Guillaudeu, R. L., and Fazekas, J. F., *Circulation Research*, **5**, 34 (1957)
360. Green, H. D., and Denison, A. B., *Circulation Research*, **4**, 565 (1956)
361. Munck, O., and Lassen, N. A., *Circulation Research*, **5**, 163 (1957)
362. Holmes, R. L., Newman, P. P., and Wolstencroft, J. H., *J. Physiol. (London)*, **140**, 236 (1958)
363. Whisnant, J. P., Millikan, C. H., Wakim, K. G., and Sayre, G. P., *Am. J. Physiol.*, **186**, 275 (1956)
364. Horvath, S. M., Kelly, T., Folk, G. E., Jr., and Hutt, B. K., *Am. J. Physiol.*, **189**, 573 (1957)
365. Johnstone, F. R., *Am. J. Physiol.*, **185**, 450 (1956)
366. Bergstrand, I., and Ekman, C. A., *Acta Radiol.*, **47**, 257 (1957)
367. Johnstone, F. R., *Surgery*, **41**, 958 (1957)
368. Selkurt, E. E., Scibetta, M. P., and Cull, T. E., *Circulation Research*, **6**, 92 (1958)
369. Trapold, J. H., *Circulation Research*, **4**, 718 (1956)
370. Schwartzkopff, W., and Pickert, H., *Klin. Wochschr.*, **34**, 255 (1956)
371. Soulie, P., Leger, L., and Sicot, J. R., *Presse méd.*, **64**, 319 (1956)
372. Gilsanz, V., Vergara, A., and Estella, L., *Arch. Internal Med.*, **100**, 201 (1957)
373. Jackson, F. C., and Happel, J. L., *Surg. Forum, Proc.*, **7**, 30 (1956)
374. Graf, K., Golenhofen, K., and Hensel, H., *Arch. ges. Physiol.*, **264**, 44 (1957)
375. Bock, H. E., Graf, K., and Hensel, H., *Klin. Wochschr.*, **35**, 487 (1957)
376. Horvath, S. M., Farrand, A., and Larsen, R., *Arch. Surg.*, **74**, 655 (1957)
377. Grayson, J., and Mendel, D., *J. Physiol. (London)*, **136**, 60 (1957)
378. Reecker, G., *Arch. ges. Physiol.*, **262**, 37 (1955)
379. Sarnoff, S. J., Braunwald, E., Welch, G. H., Jr., Case, R. B., Stainsby, W. N.: and Macruz, R., *Am. J. Physiol.*, **192**, 148 (1958)
380. Braunwald, E., Sarnoff, S. J., Case, R. B., Stainsby, W. N., and Welch, G. H., Jr., *Am. J. Physiol.*, **192**, 157 (1958)
381. Sabiston, D. C., Jr., and Gregg, D. E., *Circulation*, **15**, 14 (1957)
382. Heidenreich, O., and Schmidt, L., *Klin. Wochschr.*, **34**, 477 (1956)
383. Heidenreich, O., and Schmidt, L., *Arch. ges. Physiol.*, **263**, 315 (1956)
384. Schreiner, G. L., Berglund, E., Borst, H. G., and Monroe, R. G., *Circulation Research*, **5**, 562 (1957)
385. Bartels, H., Bucherl, E., Mochizuki, M., and Niemann, G., *Arch. ges. Physiol.*, **262**, 478 (1956)

- 386. Hensel, H., and Bender, F., *Arch. ges. Physiol.*, **263**, 603 (1956)
- 387. Davis, D. L., and Hertzman, A. B., *Ann. Otol., Rhinol., & Laryngol.*, **66**, 622 (1957)
- 388. Edholm, O. G., Fox, R. H., and Macpherson, R. K., *J. Physiol. (London)*, **134**, 612 (1956)
- 389. Roddie, I. C., Shepherd, J. T., and Whelan, R. F., *J. Physiol. (London)*, **134**, 444 (1956)
- 390. Kundt, H. W., Brück, K., and Hensel, H., *Arch. ges. Physiol.*, **264**, 97 (1957)
- 391. Green, H. D., Howard, W. B., and Kenan, L. F., *Am. J. Physiol.*, **187**, 469 (1956)
- 392. Coles, D. R., Kidd, B. S., and Patterson, G. C., *J. Physiol. (London)*, **134**, 665 (1956)
- 393. Lilienfield, L. S., Rose, J. C., and Porfido, F. A., *Circulation Research*, **5**, 64 (1957)
- 394. Ochwaldt, B., *Arch. ges. Physiol.*, **265**, 112 (1957)
- 395. Nedeljkovic, R., *Arch. intern. physiol. et biochem.*, **64**, 34 (1956)
- 396. Langston, J. B., and Guyton, A. C., *Am. J. Physiol.*, **192**, 131 (1958)
- 397. Waugh, W. H., and Hamilton, W. F., *Circulation Research*, **6**, 16 (1958)
- 398. Reynolds, S. R. M., *Am. J. Anat.*, **98**, 97 (1956)
- 399. Reynolds, S. R. M., *N. Y. State J. Med.*, **56**, 1809 (1956)
- 400. Handler, J. H., *J. Physiol. (London)*, **133**, 202 (1956)
- 401. Adams, F. H., and Lind, J., *Pediatrics*, **19**, 431 (1957)
- 402. Monkus, E. F., and Reineke, E. P., *Am. J. Physiol.*, **192**, 268 (1958)
- 403. Cobbold, A. F., and Lewis, O. J., *J. Physiol. (London)*, **133**, 472 (1956)

BLOOD CLOTTING: THE PLASMA PROCOAGULANTS^{1,2}

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Since Mann's review two years ago (1), the number of papers on blood coagulation which have appeared suggests an autocatalytic reaction, similar to the autocatalytic production of thrombin. Nearly 1200 separate publications, from late 1956 to early 1958, were considered in the preparation of this summary of recent blood clotting literature. The difficulty of commenting upon such a large and diverse group of papers led your reviewer to limit consideration to only a small segment of the recent literature, that dealing with the plasma procoagulants and related circulating anticoagulants. Many other reviews of the blood coagulation literature have appeared recently and many of the omissions may be found in them. The following reviews appear worthy of note: two general reviews of blood coagulation by Macfarlane (2) in 1956 and by Biggs & Macfarlane (3) in 1957; a monograph on hemorrhagic diseases by Quick in 1957 (4); an international symposium on hemophilia and the hemophiloid diseases, edited by Brinkhous, in 1957 (5); a review of the inherited hemostatic disorders by Brinkhous, Langdell & Wagner in 1958 (6); two reviews of hemorrhagic disorders, one by Schulman & Smith in 1957 (7), the other by Ratnoff in 1958 (8); a thesis devoted to intermediary reactions preceding prothrombinase formation by Hjort in 1957 (9); an extensive monograph on anticoagulants by Perlick in 1957 (10); a review of the chemistry of the conversion of fibrinogen to fibrin by Scheraga & Laskowski in 1957 (11); two reviews of fibrinolysis by Astrup in 1956 (12, 13) and the proceedings of a conference on fibrinolysin and proteolytic enzymes published by the New York Academy of Sciences in 1957 (14); and a monograph on thromboelastography by De Nicola in 1957 (15). In addition, an English translation by Hartmann & Guenther of Morawitz's classic paper on the chemistry of blood coagulation appeared in 1958 (16). A new international journal, *Thrombosis et Diathesis Haemorrhagica*, is now in its second volume. The annotated bibliographies of the coagulation literature by Koller (17, 18) are now appearing in this new journal.

The era of confusion which existed for many readers of the blood coagulation literature a few years ago appears to be largely past. As Carter (19) recently commented, an understanding of the current literature requires "considerable background knowledge of coagulation mechanisms. As with any discipline, this can be acquired with study; the excuse of confusing terminology wears a bit thin." Common names and probable synonyms can be

¹ The survey of literature pertaining to this review was concluded late spring, 1958.

² Among the abbreviations used in this chapter are: AcG (accelerator globulin); AHF (antihemophilic factor); PTA (plasma thromboplastin antecedent); PTC (plasma thromboplastin component); SPCA (serum prothrombin conversion accelerator); TAME (tosyl-arginine-methyl-ester); TG (thromboplastin generation).

found in several reviews (1, 5). Numerous discussions (20 to 24) have appeared in the world literature during 1957 urging that a common terminology be adopted, in line with the objectives of the International Committee on Nomenclature of Blood Coagulation Factors (25, 26). The tendency today is to employ a double nomenclature using on the one hand a descriptive term in relation to the experiments reported and on the other hand a non-descriptive code number in Roman numerals [e.g., antihemophilic factor (Factor VIII)]. Many descriptive terms are used for the same activity or factor but only the common ones will be used in this review. Some problems in nomenclature still remain. One problem in the past biennial period was posed by the assumption that certain activities described by different workers were synonymous, whereas in fact they were not. Thus the new Stuart factor activity (27, 28) was once believed to be the same as SPCA or proconvertin (Factor VII). The use of only the Roman numerals without a descriptive term may create problems. In themselves these numerals are nonmeaningful. In addition, several papers were encountered in the course of reviewing the current literature in which apparent errors had crept in, for example, Factor VII being used for Factor VIII.

The plasma procoagulant factors consist of prothrombin, along with a group of six and possibly more factors which contribute directly or indirectly to the conversion of prothrombin to thrombin. Although thrombin itself appears to have a key role in prothrombin conversion, it is not included in the group. Knowledge regarding the various procoagulants is at radically different stages of development. Prothrombin is better understood chemically than any of the others in this group. At the other extreme, doubt is expressed as to the existence of certain of the procoagulant factors which have been proposed.

Much of the recently acquired information regarding the procoagulant factors has come from the study of "bleeders" and of acquired hemorrhagic states. These abnormal biological systems provided by nature have many advantages in the study of clotting mechanisms, since certain artifacts of the test tube may be avoided with their use. Nonetheless, there are complications in the use of blood and plasma from "bleeders". One has to do with the identity of factors being studied. This difficulty has been overcome in part by the many exchanges of abnormal plasmas, and even of patients, between laboratories with the result that a number of confusing discrepancies in the literature have been eliminated. The development of circulating anticoagulants in these patients may be another complication. These inhibitors may interfere with the specificity of assay procedures. Because of the many difficulties that have been encountered, it has been proposed that the identity of a procoagulant other than prothrombin can be established with certainty only by transfusing an adequate amount of the purified material into a patient with an uncomplicated single deficiency, and observing a correction of both defective coagulation and impaired hemostasis. Obviously, this criterion is seldom met. Nevertheless, the observation serves to emphasize the need for critical appraisal of methodology at every step.

ANTIHEMOPHILIC FACTOR (AHF) AND HEMOPHILIA

AHF refers to the procoagulant plasma protein which possesses the property of correcting both the clotting defect and the hemostatic defect in true hemophilia (hemophilia A of European writers). This protein has many physicochemical properties similar to fibrinogen, and like fibrinogen it is not readily adsorbed onto such substances as BaSO_4 . AHF is known by a variety of names including antihemophilic factor A, antihemophilic globulin, Factor VIII, (alpha-)thromboplastinogen, and thromboplastic plasma component (TPC) (1). Platelet cofactor I is believed to have AHF activity (29).

Assay of AHF.—A test system specific for AHF is essential for the determination of this factor. A test sensitive to small increments in AHF concentration is also desirable. The different assays proposed attain these two objectives to a variable degree. With proper precautions those assays based on the shortening of clotting of a hemophilic substrate, either hemophilic blood or plasma, appear most reliable. The older procedures using a hemophilic substrate include the prothrombin utilization test (30) and the partial thromboplastin time (31). Newer procedures employing a hemophilic substrate utilize such clotting tests as serum prothrombin time (32), clotting time of recalcified citrated hemophilic plasma (33), and the thromboplastin generation (TG) test (34). The TG test was revised by Biggs (34) since the original test was not specific for AHF. The modification consists of using a mixture of varying proportions of hemophilic or test plasma and normal plasma in the plasma phase of the test. Plasmas which are adsorbed by $\text{Al}(\text{OH})_3$ before testing may lose some of their AHF (35). An artificial hemophilic substrate has been obtained from the first supernatant in Kekwick's cold ether procedure for fractionating plasma proteins. It has been used as a substrate in a new AHF assay procedure by Wolf (36). Many other assays, probably less specific, do not use a hemophilic substrate. These include modified tests based on the TG test (37, 38), on thrombin generation (39), or on a partial thromboplastin time determination (40). The TG test has been used widely. Miale and co-workers have analyzed a number of problems which arise if this procedure is used as a specific quantitative test (41, 42, 43). In other assay procedures purified reagents are used instead of a hemophilic plasma substrate (44, 45). These procedures also may lack specificity (29). From the many and varied methods used for AHF assay, it is obvious that there are many technical difficulties (46). In summary, those assays in which a hemophilic substrate is employed, along with a check against *in vivo* effectiveness of active material in hemophilic subjects, appear to give the most reliable results. Results of assays are usually expressed in per cent activity in comparison to a known standard, usually an "average" normal plasma. Only comparative clotting times obtained with the special test employed may be given (32). Several different units of AHF have been proposed (36, 44, 47, 48).

Preparation of AHF concentrates.—Several general procedures or a combination of procedures for fractionating plasma proteins have been used for

purification of AHF (50, 51, 58). The acid precipitation technique, using adsorbed plasmas, yielded preparations 4 to 8 times concentrated (51). The ethanol-low temperature technique has usually given low yields and weak preparations of Fraction I, although some effect has been observed on *in vivo* administration to hemophiliacs (52, 53). Greatly improved preparations with the ethanol precipitation procedure have been obtained by using fresh plasma, specially adjusting the alcohol concentration and ionic strength, and selectively extracting the precipitates. Human (1, 44, 54, 55, 56), bovine (57), and canine plasmas (35, 58) have all been used as starting material. Many preparations are 5 to 20 times concentrated, and a few are as much as 100 times concentrated. The low temperature-ether fractionation procedure has yielded a human AHF preparation approximately 20 times concentrated, suitable for therapy (59). Salting-out procedures have yielded the most potent preparations, particularly from animal plasmas. Platelet cofactor I has been highly purified with only a single peak in the ultracentrifuge (60); it was purified by a combination of kaolin adsorption, elution, alcohol precipitation, and salting out procedures. Unlike other preparations it withstands ether extraction. This preparation had a high carbohydrate content, including amino sugar(s). Bidwell's technique (61) for AHF purification is particularly effective. In our laboratory (50, 58) a porcine plasma fraction 1600 times purified has been obtained which is roughly 8 to 12,000 times as potent as human plasma. On the basis of the purity of the best preparations, it is calculated that AHF is a trace protein with a level of less than 5 mgm. per cent in plasma (50). A preparation so low in protein was obtained by van Creveld and co-workers that they questioned whether AHF is actually a protein (54).

AHF and fibrinogen usually separate together from plasma. A fibrinogen-free AHF preparation was first prepared from canine plasma by Wagner and co-workers (62). This procedure has been studied further and best conditions for its preparation delineated (35). Unfortunately this method for separating AHF and fibrinogen does not appear applicable to plasmas of other species. Recently several other methods have been devised for separating AHF and fibrinogen. These have been applied to a number of mammalian plasmas as well as to human plasma. In one procedure, $\text{Al}(\text{OH})_3$ adsorption removed the fibrinogen but left much of the AHF in solution (35). Other methods include heat precipitation of fibrinogen (54), glycine extraction of a bovine or human Fraction I precipitate (56, 57), combined alcohol-ether fractionation (44), or Walton's procedure for precipitating fibrinogen with high molecular weight dextran sulfate (63).

Properties of AHF.—Ultracentrifugal studies indicate that AHF sediments more rapidly than fibrinogen, suggesting that it has a high molecular weight (58). AHF cannot be passed through filters successfully, perhaps because of large molecular size or protein-protein interactions. Seitz filtration removes AHF, yielding an artificial hemophilic plasma (35). Because of low solubility and irreversible adsorption onto paper, electrophoretic patterns have been difficult to attain (54, 64). Different workers have shown it to

migrate with both beta and alpha globulins. The most highly purified preparations have a typical protein ultraviolet adsorption curve (58). AHF is inactivated by heating at 56°C. for 10 min. (56, 65). At somewhat lower temperatures, e.g., 3 hr. at 49°C., inactivation is incomplete and an anomalous denaturation curve is observed (58). Purified bovine and porcine AHF preparations appear to be antigenic for man and rabbits. They cause thrombocytopenia on injection, and agglutinate human platelets *in vitro* (47, 48).

Stability of AHF.—Recent studies, in contrast to much data in the earlier literature, indicate that AHF is comparatively stable in plasma if proper precautions are taken in the collection and storage of blood (65, 66). Penick and co-workers have shown that AHF, after some initial losses, is relatively stable in frozen or lyophilized plasma (65, 66). Rapid freezing preserves more AHF than slow freezing (56). In carefully prepared liquid and frozen plasmas almost all the AHF is retained for three weeks, and in lyophilized plasma AHF is stable for years (65). AHF is particularly stable in platelet-free frozen plasma (36). Thrombocytopenic bloods are more stable on storage, and rate of loss of thromboplastic potency parallels disruption of platelets (67). Diluted plasmas also appear to be more stable in respect to AHF (37). Penick has shown that the most important factor in maintaining AHF in plasma appears to be removal of a source of thrombin, or prevention of prothrombin conversion (68). AHF is stabilized in plasma by BaSO₄ adsorption. Careful collection of blood (65), prompt decalcification including magnetic stirring to obtain prompt mixing with anticoagulant (56), and removal of prothrombin (68) all appear to be as important as storage temperature, AHF level of the donor, or type of anticoagulant. Heparin appears to prevent inactivation of AHF by thrombin (69). Storage of blood or plasma at pH 6.1 to 6.5 in a carbon dioxide atmosphere stabilizes AHF even at room temperature (70) although it was found to be unstable at similar pH values if disodium citrate was used as an anticoagulant (67). Also, Blombäck's preparation was unstable if the pH was kept at 6.0 (56). The instability of AHF in oxalate solutions is well-established (65). This fact may account for the sensitivity of the TG test to even short periods of storage of one of the reagents in the test, oxalated adsorbed plasma (41).

Metabolism of AHF.—The organ or tissue of origin of AHF remains obscure. Splenectomy does not influence the clotting function of blood of either canine or human hemophiliacs (71). In contrast to older data on hepatotoxic damage and AHF, Penick and co-workers (118) showed that moderate depression of plasma AHF follows severe chloroform damage to the liver. Whether this is related to increased utilization of AHF in the presence of necrosis of liver tissue, or to impaired production if the liver is damaged is not yet determined. A number of studies have confirmed the observation made by our group several years ago that injected AHF disappears rapidly from the plasma (56, 66, 72, 73). Recent studies have been made on both hemophilic dogs and on hemophilic patients, using transfusions of either normal plasma or plasma fractions rich in AHF. AHF is lost rapidly from the blood stream, roughly one-half of the clotting factor disappearing in 6 to 12

hr. (34, 66, 72, 73, 74). Wagner and co-workers have extended these studies by injecting potent AHF fractions intravenously, subcutaneously, and intramuscularly into hemophilic dogs (72). If AHF was maintained at high levels for many hours, then the rate of fall-off was found to be relatively slow. The plasma AHF level fell to a one-half value in about 24 hr. The very rapid loss of AHF following single injections appears to be accounted for in part by equilibration between vascular and extravascular compartments. Brinkhous & Penick (75) showed that AHF is utilized more rapidly in the presence of tissue necrosis, and Langdell (76) pointed out that in hemophiliacs with spreading hemorrhages and tissue damage, increasing amounts of AHF (a "vicious cycle") are required to maintain a given plasma level. Blombäck & Nilsson (74) have shown that infection also increases AHF requirements. These findings may account for the disasters in hemophilic patients with hemorrhagic crises which occur despite transfusion therapy, as well as the varying amounts of AHF required to maintain a given AHF level in different subjects (48). Direct evidence of the local binding of AHF in the fibrin of wounds was provided by studies with fluorescein-labelled AHF (47).

Genetic control of AHF.—Genetic studies on AHF deficiency suggest that there is a locus on both the X-chromosome and one of the somatic chromosomes which is concerned with AHF fabrication (33, 77, 78). Graham has reviewed the biochemical genetic data (77). The sex-linked recessive inheritance of classical hemophilia has been repeatedly confirmed. The multiple allele hypothesis of X-chromosome inheritance was proposed (79) to explain the varying grades of hemophilia (severe, moderate, mild, and subclinical) as well as the AHF levels in normal individuals which, while constant for a given individual, vary from about 50 to 180 per cent of the mean of a normal population. This hypothesis has been reconsidered by Graham (77) in relation to certain heterozygotes in a pedigree of mild hemophiliacs who have low AHF values, and in relation to females with mild hemophilia. Modifying genes at other than the hemophilic locus on the X-chromosome may account for some of the aberrations observed. While female hemophilic dogs are common in our laboratory, human female hemophiliacs, while once considered nonexistent, are being reported. A few appear to be homozygotes (80) but the majority appear to be heterozygotes with marked expressivity (81, 82 to 85), comparable to those in the original pedigree described by Brinkhous & Graham (79). In spite of this, the majority of carriers appear to have normal AHF levels (86, 87, 88). The broad range of normal AHF values, originally reported by Langdell and co-workers (31) has been confirmed by several authors (34, 37, 86). The importance of an autosomal genetic locus for AHF fabrication is emphasized by the many reports of vascular hemophilia (33, 78, 89 to 98) in which there is a moderate reduction in plasma AHF along with a prolonged bleeding time. The pattern of inheritance of this variant of hemophilia appears to be autosomal and dominant. Many cases of von Willebrand's disease in which there is a moderate depression of plasma AHF levels appear to be in this same category (91, 99 to 104).

Role of AHF in clotting and hemostasis.—AHF is an essential factor for

prothrombin utilization in clotting blood and for effective hemostasis. The minimum level of AHF required for effective hemostasis in the presence of hemorrhage has been estimated variously from as low as about 5 to 10 per cent of normal to as much as 50 per cent of normal (48, 56, 66, 76, 105). Recent estimates have been 10 to 15 per cent (56) and 30 per cent (48) of normal respectively.

Numerous different suggestions have been made regarding the place of AHF in the clotting mechanism. It is most frequently suggested that it acts jointly with platelets, particularly the thromboplastic lipide portion (e.g., threonine system of Seegers). Wolf, however, has questioned if AHF is a platelet cofactor (36), as might be deduced from the old data of Brinkhous (106). A Flynn-Coon type of sedimentable thromboplastic material has been observed to form from an incubated mixture of serum, AHF-containing plasma, and calcium, followed by platelet addition. Bergsagel & Hougie have called this Product I (107). It was believed that AHF combined with Christmas factor. It has been suggested that AHF participates in the formation of the clotting intermediate, convertin (108, 109). It has also been suggested that AHF interacts with AcG and thrombin to form a prothrombinase enzyme (110); perhaps this enzyme is thrombin itself (111). Others have questioned if AHF participates in a preliminary reaction (112). Activation of Hageman factor appears to be independent of AHF (113).

The old finding of Graham *et al.* (114) that AHF is consumed during clotting has been repeatedly confirmed (68, 115). Penick (68) demonstrated that AHF consumption occurs only if thrombin or a source of thrombin, i.e., prothrombin, is present in the plasma. Calcium accelerates the AHF-inactivating action of thrombin. Plasmas with impaired clotting ("dicumarol" plasma, Christmas disease plasma, heparinized plasma) also have impaired AHF consumption (115). Present data strongly suggest that AHF is a substrate for thrombin. While a more active coagulant may be formed from AHF, this is not established. It is proposed that the thrombin-AHF system may function at times as a negative feedback mechanism (116).

It has been confirmed by Brayton & Zucker (117) that thrombin injected intravenously will cause a severe reduction in plasma AHF, along with a hypofibrinogenemia. Penick and co-workers (118) have shown that a similar depression of plasma AHF follows injections of thromboplastic tissue extracts; if the animal has an impaired clotting mechanism before thrombin is injected, this effect on AHF is reduced or eliminated, comparable to the *in vitro* data, suggesting that anything interfering with thrombin formation will spare the antihemophilic factor.

Pathogenesis of hemophilia.—A preponderance of presently available data supports the thesis that hemophilia is caused by a deficiency of plasma AHF. Other data suggest that an abnormality in platelets may exist or that the basic defect is due to an excess of an anticoagulant. The platelet data are conflicting. No difference was observed in the coagulant activity of normal and hemophilic platelets (119). However, in Mann's co-thromboplastin test, washed hemophilic platelets caused subnormal coagulant activity (120).

Fonio observed that hemophilic platelets were more stable during clotting than were normal platelets (121).

A new inhibitor, Bridge anticoagulant, has been described in both hemophilia and Christmas disease (122, 123, 124). This concept has been questioned, with the suggestion that quantitative differences in AHF levels in the tests rather than a previously unrecognized inhibitor were responsible for the results (125). Other data have supported the old inhibitor-excess hypothesis of the nature of hemophilia. The threone combination of Seegers failed to correct the clotting time of some grades of hemophilia (29). Carroll *et al.* (126) found that transient "hemophilia" could be produced in normal and thrombocytopenic patients by transfusions of Grade IV hemophilic blood. Re-examination of some of the basic experiments on which the inhibitor-excess hypothesis was originally based has led to alternate interpretations of certain of the findings. Graham & Barrow (127) suggested that the biphasic curve of clotting (slow clotting in both undiluted and highly diluted plasmas) results from variations in ionic strength rather than a high inhibitor level in undiluted plasma. Clotting time of hemophilic plasma was "normalized" by treatment with asbestos, glass, or kaolin (113, 127, 128). The "normalized" asbestos-treated plasma failed, on transfusion into hemophilic dogs, to correct the clotting abnormality (127). These findings suggest that a "glass" or Hageman factor was activated rather than that an inhibitor removed, as was once thought. Ether treatment of plasma caused both activation of an accelerator and inactivation of AHF (127). Ether-treated plasma is inactive in the TG test, and ether-containing AHF fractions lose activity (36).

Hemophilia and vascular factor.—Vascular hemophilia and many cases of Von Willebrand's disease as well as pseudohemophilia B have a long bleeding time associated with a mild deficiency of AHF, or occasionally of Christmas factor. It has been confirmed that the long bleeding time is corrected temporarily by blood or plasma transfusions (33). The factor in plasma responsible for this correction has been found to be associated with AHF in Fraction I-O by Blombäck & Nilsson (33, 99). They have termed this plasma component vascular factor.

Miscellaneous.—Hemophilia is the commonest of the inherited hemorrhagic diseases, accounting for 40 to 90 per cent of bleeders in different studies (77, 129 to 132), with an estimated frequency of 373 per million males (77). About 25 to 50 per cent of cases give no family history of the disease (39, 130 to 134). About one-fourth of the cases encountered are mild clinically and have a normal clotting time (39, 132).

Complement titers in hemophilic and hemophilioid disease sera have been found to be normal (135). The electrophoretic pattern of hemophilic plasma has been variously claimed to be normal, to show a high level of gamma globulin without abnormal components (136), or to contain an abnormal component (137). The diagnosis of hemophilia and its differentiation from hemophilioid disease by clotting tests have been considered in a large number of publications (32, 39, 131, 138 to 150).

Material possessing AHF-like activity in the TG test has been isolated from tissues (151, 152). Hemophilic saliva possesses normal thromboplastic activity (153).

Circulating anticoagulants acting against AHF.—Refractory hemophilia, i.e., hemophilia which no longer responds to plasma transfusions, was the subject of many reports. It is not certain that the inhibitors which complicate hemophilia are qualitatively all the same. The antigenicity of AHF in administered blood, plasma, or plasma fractions may account for some of the inhibitors observed. Richards & Spaet (154) demonstrated that a human AHF preparation was antigenic for the rabbit. The rabbit antibody possessed inhibitor properties. Others have failed to demonstrate an antibody in serum of patients having a circulating anticoagulant (155, 156, 157). However fractions obtained after zone electrophoresis with high inhibitor activity did give an antibody-antigen reaction (155). The problem of false positive precipitin reactions has been discussed (133, 156, 158). A number of additional patients with refractory hemophilia have been described (159, 160, 161). Circulating anticoagulants that appeared to have an anti-AHF action have also been described in nonhemophilic subjects (128, 160, 162, 163, 164, 251). The case reported by Nilsson and co-workers (163) developed in the post-partum period without transfusions and responded to ACTH. The inhibitor was in the gamma globulin fraction. It interfered with thromboplastin generation and could be neutralized by adding excess AHF. Evidence of a lipid inhibitor occurring in hemophilia was presented by Speer and co-workers (165).

PLASMA THROMBOPLASTIN COMPONENT (PTC) AND CHRISTMAS DISEASE

PTC is also known as Christmas factor, Factor IX, autoprothrombin II, and beta prothromboplastin (2). PTC is recognized by its ability to correct the clotting defect in subjects deficient in this plasma procoagulant. The inherited deficiency disease is termed variously Christmas disease, PTC deficiency, or hemophilia B. Blood or plasma in this disease is characterized by a normal prothrombin time, a long partial thromboplastin time (166), impaired prothrombin consumption, and an abnormal thromboplastin generation test, serum phase. The active principle is present in plasma and serum as well as in BaSO₄ eluates of plasma and serum, but not in adsorbed plasma or serum. Rabbit blood contains the principle in the same titer as human blood (167). Considerable difficulty may be encountered in qualitative recognition of Christmas disease. Not only patients with PTA deficiency (168), Stuart factor deficiency (133), and Hageman factor deficiency (169, 170) but also normal subjects (171) have been mistakenly diagnosed as PTC deficiency. Conversely, a patient thought to be deficient in a new factor was found to have a mild deficiency of PTC with an inhibitor (172). Rodman and co-workers (166) have emphasized the necessity of using cross-matching tests with a known deficient plasma for the identification of PTC deficiency.

It has been suggested that other abnormalities accompany PTC deficiency. An excessive amount of inhibitor may be present (123, 173). A platelet abnormality has also been suggested (174).

Assay and purification of PTC are not as far advanced as is the case for AHF (175). Attempts to measure PTC have been made by serial dilution of sera in the TG test (176). This approach is complicated by the presence of other BaSO_4 -adsorbable procoagulants in serum, particularly Stuart factor, which will affect the test. This fact may account for the suggestion that PTC and proconvertin are identical (177).

Much circumstantial evidence is accumulating to indicate that the metabolism of PTC, like that of prothrombin, proconvertin, and Stuart factor, is dependent upon proper hepatic function and adequate vitamin K. PTC is depressed when the coumarin-indanedione drugs are administered (178 to 184). PTC responds to vitamin K_1 administration, but a longer time is required for restoration of normal PTC levels than is the case with prothrombin and proconvertin (81). Like prothrombin, PTC in the plasma of newborns and of infants is normally low (185, 186, 187, 314), although a contrary report has appeared (176). This factor is also depressed in some cases with acute and chronic liver disease (179, 180, 181, 188, 189) as well as with uremia (181). In evaluating these reports attention needs to be given to the specificity of the tests employed. Little has been added in the biennium just past regarding physical and chemical properties of PTC (175). A plasma fraction 10 to 20 times concentrated has been reported (175, 190, 191). PTC may be a nonthrombic derivative of prothrombin (173). PTC is not reduced by intravenous injections of thrombin (117).

PTC fabrication in the body appears dependent on a gene on the X-chromosome, at a locus different from the gene related to AHF production. The sex-linked character of the inheritance of PTC deficiency in the famous Tenna bleeders has been restudied in detail (124, 192, 193). Hemorrhagic manifestations with lowered PTC levels continue to be reported in heterozygotes (88, 175, 189, 192, 194, 195) but this finding is not sufficiently consistent to be a reliable means of identifying transmitters of the disease (175).

Recent studies on the stability of PTC in stored blood, plasma, and serum have failed to confirm the thesis that PTC in serum is more effective *in vivo* than is PTC in plasma (196, 197, 198). Plasma stored for five days at 4°C . appears to have a high content of PTC (196), while blood stored for 14 days under blood bank conditions has been reported to have little activity (198). Plasma several months old has been mentioned by Brinkhous as completely inactive (199).

Circulating anticoagulant acting against PTC.—The inhibitor which occurred in one of the early cases of Christmas disease was shown to act against PTC (160). This inhibitor was associated with a great increase in gamma globulin (35 per cent of total proteins). As the titer of the inhibitor decreased, the gamma globulin decreased (to 18 per cent). Similar patients with Christmas disease complicated by inhibitors have been described (161, 200, 201). Fantl and associates suggested that two types of PTC deficiency occur, the

one with a complete lack of this protein, the other with a protein having antigenic properties similar to PTC but functionally inactive in the clotting system. Only the first type would develop circulating anticoagulants against PTC. A human serum preparation containing multiple activities, including PTC, was found to be antigenic for the rabbit (202).

ACCELERATOR GLOBULIN (AcG) AND PARAHEMOPHILIA

Fantl (203) has prepared an excellent review of the literature on this clotting factor. Common synonyms for AcG are labile factor, proaccelerin, and Factor V. For a complete list of presumed synonyms see Fantl (203). The inherited deficiency state is relatively rare and is known as parahemophilia. The mode of its inheritance has been reviewed (77, 203, 204); seriously affected individuals appear to be homozygotes, while all offspring show some reduction in this factor. Both an autosomal recessive and a partially dominant pattern of inheritance have been suggested. Larrieu (205), Lewis (206), and their co-workers have reviewed the congenital cases of AcG deficiency, and an additional case has been added (207). With a deficiency of this factor there is characteristically a long prothrombin time, a long partial thromboplastin time (166), poor thromboplastin generation (208), and impaired prothrombin utilization. The presence of AcG activity in normal platelets and its absence in platelets of patients with parahemophilia have been confirmed (207, 209).

For the assay of AcG an aged plasma artificially deficient in AcG is frequently used. The anticoagulant is either oxalate or ethylenediaminetetraacetic acid (EDTA). Two different methods for obtaining a severely deficient plasma quickly by controlled incubation at 37°C. have been reported (210, 211). Asolectin, a soya bean phospholipide, binds this procoagulant (212).

Although AcG under ordinary circumstances is very labile, it can be stabilized if proper precautions are taken. It is most stable if the blood used for its preparation is promptly decalcified and kept in the pH range of 6.4 to 7.0 (203, 213). The presence of manganous, zinc, nickel, or barium ions appears to stabilize AcG also (214). AcG is lost rapidly in EDTA plasma; this loss is partially prevented by magnesium, barium, calcium, or strontium ions (215).

A number of studies on species differences of AcG have been made. This procoagulant has been found in the blood and lymph of all species studied (203). A comparative study of plasma AcG showed the dog to have the highest level, cow and sheep intermediate levels, and human the lowest value (213). After activation with thrombin, serum AcG activity was in the reverse order. Species differences in adsorbability onto BaSO₄ were observed with bovine and human sera (216). AcG in horse plasma was readily adsorbed onto BaSO₄ (213).

AcG appears to have a half life of about 20 hr., as judged by the rate of loss of AcG injected into parahemophiliacs. A level at least 30 per cent of normal seems to be needed for effective hemostasis (203). Splenectomy does

not affect AcG (71), and thrombin injections cause only slight reduction in this factor (117). It is increased for a short time following estrogen administration (217). Carter & Warner (218) showed in an important study that reduced AcG levels may under certain circumstances respond to vitamin K plus methionine, or to vitamin K-S(II).

Fresh and co-workers indicate that plasma levels are above normal in the newborn (186), a finding which contrasts with earlier studies. An interesting study was made of a parahemophilic female during the course of pregnancy (219). A normal child was delivered. Apparently AcG was not transferred across the placenta to the mother. The mode of action of AcG remains unknown, but it is suggested that it affects the rate of TG (207). On the other hand, the increased coagulant activity that accompanies TG is believed by Seegers to represent the conversion of plasma AcG to serum AcG (220).

A chromatographic method for purification of AcG, using Amberlite resin has been reported by Cox, Lanchantin & Ware (216). Human plasma exposed to Amberlite IR-400 loses little activity, while human serum activity is adsorbed and can be eluted. This technique has resulted in about a 1000-fold purification. The major portion of the AcG activity was found to migrate as a single component between the gamma and beta globulins. AcG is stable with thrombin, unlike AHF (69).

Circulating anticoagulants acting against Factor V.—A well-documented study of an anti-AcG inhibitor which appeared spontaneously in a patient who had no history of a hemorrhagic diathesis (221, 222) has been made by Ferguson, Johnston & Howell. The anti-AcG inhibited thromboplastin generation in plasma as well as prothrombin conversion. Thus AcG may be required in several clotting reactions. The partial thromboplastin time of hemophilic, aged, Stuart-deficient, and PTC-deficient plasmas was not corrected by the patient's plasma. Because of these findings the authors point out the need for cautious interpretation of all clotting tests when a circulating anticoagulant is present.

STUART FACTOR AND STUART FACTOR DEFICIENCY

This factor is a newcomer to the procoagulant group, having been dissociated from Factor VII in 1956-57 by Hougie, Barrow & Graham (27, 223). These authors reviewed previously reported cases of Factor VII deficiency and pointed out that while the prothrombin time test was abnormal in all of the patients, there were two patterns of reaction with the TG test and prothrombin consumption tests: in one group these tests were normal, in the other group they were abnormal. Restudy of a patient named Stuart (27) who previously had been characterized as having hypoproconvertinemia showed that there was mutual correction of the prothrombin time prolongation on mixing plasmas of this patient and the prototype case of SPCA deficiency described by Goldstein & Alexander (224). Graham and associates proposed that the missing factor in their patient be named Stuart factor. Absence of Stuart factor is characterized by a long prothrombin time, abnormal thromboplastin generation, impaired prothrombin utilization, a long

partial thromboplastin time, and a long recalcified clotting time (27). Russell's viper venom, substituted for tissue thromboplastin, does not correct the long prothrombin time (27, 225). Several patients, some previously classified as Factor VII deficiency, SPCA deficiency, idiopathic hypoprothrombinemia, or stable factor deficiency, were found to have Stuart factor deficiency (27, 226 to 230, 315). Other cases appear to be in this same category (131). Factor X activity appears to be in part dependent on Stuart factor (229). It has been suggested that Stuart factor be named Factor XI (232).

Assay.—Mixing experiments with a known deficient plasma gave the greatest specificity to an assay for Stuart factor. The original assay, using the prothrombin time test, was based on this principle (27, 166). By using a Russell's viper venom (Stypven)-cephalin mixture as thromboplastin, the need for a Stuart deficient plasma may be obviated (225, 229, 233, 234). Contact with glass has no effect on this factor (234). The prothrombin time is sensitive to moderate reduction in Stuart factor (229). The TG test becomes abnormal only if Stuart factor falls below the 25 per cent level (229). In the two-stage prothrombin test, prothrombin conversion is impaired (27) unless this factor is supplied.

Metabolism.—This factor, like other BaSO_4 -adsorbable procoagulants, appears to be related to the vitamin K-dependent protein synthesizing mechanism in the liver. With administration of the dicumarol-type drugs, this factor is depressed, but more slowly than Factor VII, and more rapidly than Christmas factor (27, 229, 233, 235, 236). Stuart factor responds more slowly to vitamin K_1 (229) than does SPCA. The half life of this factor in plasma is not determined, but it appears from the limited data available to be about 24 hr. (229). Stuart factor injected into patients deficient in this factor disappeared almost completely from the plasma in five days. Judging from marcoumar-treated patients, the hemostatic level is 10 per cent of normal or higher (234). The disease, Stuart factor deficiency, appears to be inherited as a highly penetrant, but incompletely recessive, autosomal characteristic (28).

Stuart factor is adsorbed onto Seitz filters, BaSO_4 , $\text{Al}(\text{OH})_3$, and $\text{Ca}_3(\text{PO}_4)_2$. It is eluted with citrate. In plasma it is destroyed by heat at 56°C . and appears to be relatively stable on storage (27, 229, 234).

SPCA (FACTOR VII) AND ITS DEFICIENCY

Until recently it was often assumed that a single factor in serum was responsible for the accelerating effect of serum on the prothrombin time. A number of terms were commonly used for this activity and they were frequently assumed to be synonyms: serum prothrombin conversion accelerator (SPCA), Factor VII, proconvertin, and stable factor. Other terms have also been used (1, 2). It is now generally realized that these terms were describing a serum activity which could result from two or more factors. Since this fact has only recently been recognized, numerous papers appearing in the past two years are difficult to evaluate in the light of current concepts.

Assay.—It appears that any test for SPCA, to be specific, must have as its

substrate a plasma deficient only in this factor. The usual one-stage assays for Factor VII appear not to be specific for this factor, since they have been shown to be affected by changes in Stuart factor (9, 27, 224, 233). In the past, patients whose plasma clotting defect was corrected by a BaSO₄ eluate of serum as well as by a PTC-deficient plasma were believed to have a Factor VII deficiency. If in addition the partial thromboplastin time and TG test are normal (166, 237), the presumption is that Factor VII is missing. This is further fortified if a thromboplastin consisting of Russell's viper venom and cephalin corrects the long prothrombin time (166). A number of patients have been reported recently who appear to have this defect (166, 238 to 249), while other patients described in the literature probably need additional study before characterization can be definite (231, 250). The older data indicating that SPCA is decreased rapidly after administration of the coumarin-indanedione drugs and that it responds readily to vitamin K₁ appear to be substantiated by recent work. The turnover rate of this factor in the plasma appears to be rapid; half life in man appears to be in the neighborhood of 5 to 10 hr. (252).

Goldstein & Alexander (224) reported that prothrombin, in the absence of proconvertin, will not form thrombin with tissue thromboplastin, emphasizing again the "tissue thromboplastin cofactor" nature of SPCA. This same prothrombin is readily converted to thrombin if platelets are used for thromboplastin.

Other proposed procoagulants in serum.—A number of other serum clotting factors, similar in many respects to SPCA and Stuart factor, have been reported. Factor X, instead of being a single factor, may be a manifestation of combined prothrombin and Stuart factor deficiency in dicumarol-type plasma (229, 236). It is probably not synonymous with Stuart factor (232). Other factors have been suggested (253, 254), including the Car. factor (255). Sera from these patients were studied in the TG test.

HAGEMAN FACTOR AND HAGEMAN TRAIT

This factor was named for the patient, Hageman, who lacked this activity in his plasma (256). In the absence of this factor, clotting tests carried out in glass are profoundly affected (113). Hemostasis on the other hand is altered but little if at all in the absence of this factor. Hageman factor appears to be identical to the plasma clotting factor which is activated by glass contact ("glass factor", "contact factor") (64, 113, 257, 258, 259), although definitive data are needed.

Blood or plasma devoid of Hageman factor has a prolongation of the whole blood clotting time, recalcified plasma clotting time, and partial thromboplastin time (166, 260, 261) and gives an abnormal TG test (262), thrombin generation test (263), and thromboplastin screening test (168). The serum prothrombin time is abnormally short and the prothrombin time only slightly prolonged (166). Bleeding time and capillary permeability are not altered. The factor appears distinct from AHF and PTC (168, 264), as well as prothrombin, SPCA, AcG, and Stuart factor (257). However, addition of

Hageman factor to these deficient plasmas will shorten their clotting times! It also shortens the clotting time of normal blood, even in a very weak concentration.

Hageman factor is present in both human plasma and serum. It is not adsorbed onto BaSO_4 . The factor is nondialyzable (264). It has been found in the blood of the rat, mouse, rabbit, guinea pig, sheep, and dog, but is said to be absent in Peking ducks (258) and horses (170). It is relatively stable on storage of plasma (260) and is stable on heating plasma at 56°C . for 20 to 30 min. At 60°C . it is inactivated (168, 260, 264). Freezing and thawing of Hageman-deficient plasma tend to correct the defect (170). Assays for Hageman factor are based on the shortening of the clotting time of recalcified Hageman-deficient plasma (265) or of silicone plasma (266). Transfusions of normal plasma into patients with the Hageman trait correct the coagulation defect (260, 262). Electrophoretic studies by different authors indicate its migration with the gamma globulins (64) and the beta globulins (264), or between them (267). A serum fraction with a 500-fold concentration over normal serum has been reported (268). The procedure for purification of Hageman factor consists of "salting-out" the protein fraction of BaSO_4 -adsorbed normal serum between 30 and 40 per cent saturation with $(\text{NH}_4)_2\text{SO}_4$. The activity is adsorbed onto kaolin at pH 5.2, and eluted selectively with a tris buffer and ammonium sulfate. This activity is present in Cohn's Fractions III, IV-1, and IV-4 (267).

The mode of activation of Hageman factor has been the subject of several studies. Neither calcium nor platelets are needed (259, 266). A number of surfaces can substitute for glass in the activation process. Included are dried silica and alumina which have been heated to 1000°C . (259), colloiddion (269), and asbestos (257). It has been suggested by Quick that the glass activation phenomenon represents a change of prothrombinogen to prothrombin or, alternatively, that thrombin adsorbed onto glass accelerates a reaction between platelets and a plasma factor, possibly AHF (269).

A possible interaction between Hageman factor and PTA (see below) has been suggested. They may act synergistically (168). Plasmas deficient in PTA have been reported as capable of correcting Hageman-deficient plasma (267). Others report that this correction is incomplete (168, 260). The clotting of Hageman-deficient plasma is accelerated by adding the glass activated factor (270); PTA-deficient plasma appears insensitive to addition of the activated factor. Margolis proposes a two-stage reaction for Hageman factor activity to be manifest. In the first stage Hageman factor is activated by glass contact. The activated Hageman factor then acts on another precursor substance, possibly PTA (259). The active Hageman factor is believed to activate other plasma factors also, including a pain-producing factor, a uterus-stimulating factor (259), and a permeability factor (271). Another suggestion is that Hageman factor acts on a precursor of PTC, giving a material which is more active in the TG test (168). It has also been proposed that Hageman factor is activated by adsorption of an inhibitor onto glass (258). An anti-Hageman factor has been postulated, based on the decay of

the glass-activated material in plasma and serum (272). Soya bean trypsin inhibitor interferes with the action of Hageman factor.

Johnston & Ferguson (257) could not isolate this factor from Hageman-deficient plasma. They suggest that failure of a plasma to develop a more rapid clotting ability on contact with asbestos is a simple presumptive test for the absence of Hageman factor. Hageman trait is believed to be inherited as an autosomal recessive (265). Several cases of Hageman trait have been reported (166, 273, 316).

PLASMA THROMBOPLASTIN ANTECEDENT (PTA) AND PTA DEFICIENCY

This factor, a deficiency of which causes a mild hemorrhagic tendency (49), has been termed also antihemophilic factor C (274) or the third thromboplastic factor. With a deficiency of PTA the serum prothrombin time is abnormally short, the partial thromboplastin time is prolonged, and thromboplastin generation is incomplete (166, 168, 275 to 279). Prothrombin time and bleeding time are not influenced by this factor. The TG test may be normal if there is only a partial deficiency of PTA (280). Storage of plasma deficient in PTA may become activated, so that it will correct a PTA deficiency (168, 280).

PTA has been partially characterized (281, 282). It is present in the plasma protein fraction precipitated between 20 and 30 per cent saturation with $(\text{NH}_4)_2\text{SO}_4$, and migrates between the gamma and beta globulins (274, 281). BaSO_4 -adsorbed normal serum is said to contain PTA, since it is only partially adsorbed onto BaSO_4 (132). The factor is active in stored blood.

Transfusions of BaSO_4 -treated serum into a deficient patient partially corrected the impaired TG test (279). An effect lasted for about seven days, judging from changes in the patient's whole blood clotting time. However, the serum prothrombin time shows only transient correction, suggesting that larger amounts of the factor are needed to alter this test (280, 283). PTA has been reported to be decreased with avitaminosis K, dicumarol therapy, and liver injury, reacting like the BaSO_4 -adsorbable procoagulants, prothrombin, PTC, Stuart factor, and Factor VII (180).

The mode of action of PTA in the fibrin-clotting mechanism remains obscure. Margolis (259) has suggested that PTA is activated by Hageman factor after glass contact; hence PTA-deficient plasmas are not sensitive to Hageman factor (264). Biggs and co-workers (168) suggest that PTA acts after the glass-activation factor, and that active PTA in turn is needed for proper activation of Christmas factor. Several patients with PTA deficiency have been reported (283, 284), one with a circulating anticoagulant (285).

FOURTH THROMBOPLASTIC FACTOR (PTF-D)

The history of this factor was reviewed by Mann (1). It now appears not to be a separate clotting factor. The abnormal clotting observed was the result of a mild deficiency of PTC, combined with an inhibitor (172).

PROTHROMBIN

Prothrombin continues to hold the center of interest in the coagulation process, since its activation to thrombin provides the means of fibrin formation. The many other procoagulants described appear to function in preliminary reactions to make prothrombinase activity available. Measurement of prothrombin continues to be the subject of several reports. Another modification of the two-stage procedure of Warner, Brinkhous, and Smith has been made. Dreskin (286) has devised a relatively simple two-stage procedure using hemolyzed whole blood; the lysed red cells furnish the thromboplastin. A possible source of error in prothrombin determinations is the loss of this substance by adsorption onto calcium oxalate if oxalate is used as an anticoagulant (287). The TAME assay for prothrombin has been restudied; a good correlation exists between the results with this test and with the two-stage procedure (288, 289). The TAME assay is relatively independent of AcG and Factor VII content of the test materials. This assay has been used for the control of anticoagulant therapy (289). It is of interest that Seegers and co-workers (290, 291) have been able to dissociate the TAME-splitting and the fibrin-clotting activities of thrombin, a finding which raises the question if esterase-thrombin or biotrombin is the better measure of prothrombin. A micromethod for doing "prothrombin" time determinations has been reported (292). Determinations on blood appear to be valid up to 24 hr. after withdrawal from the vein (293).

Metabolism.—Several studies have been published on plasma prothrombin levels in the fetal, neonatal, and infantile period. In a Russian study, "prothrombin" time values were very long in 24-day rabbit fetuses, with a gradual shortening of values until term (294). It is suggested that the values are a reflection of the state of development of hepatic function. Fresh and co-workers (186, 187) have determined the prothrombin level in cord blood of the newborn and during the neonatal period. They found wide variations in the prothrombin levels, with adult values being reached in about 8 to 10 weeks. Little response to antenatal vitamin K administration was observed. Most of these children were fed on artificial formulae. Dyggve (295) found in breastfed infants a fall in prothrombin on the third day after birth. Prothrombin levels were somewhat higher if menadione had been administered. The prothrombin values reached adult values in 10 to 12 months, in agreement with the original data reported in 1937 (296).

A number of miscellaneous studies on prothrombin have been reported. A restudy of prothrombin levels in man, horse, ox, and dog yielded relative values of 100, 99, 86, and 80 per cent respectively (297). The old data on prothrombin levels (298) in lymph have been confirmed (299). Hypoprothrombinemia of vitamin-K deficiency is accompanied by depressed levels of Factor VII in cholecystonephrectomized dogs (300) and of PTC in patients with obstructive jaundice (301). A transient increase in plasma prothrombin occurred in dogs given estrogens (217). Prothrombin levels in the plasma appear to offer a sensitive index of hepatic function in inflammatory liver disease, judged by a correlation with the findings of liver biopsy (286).

The manufacture of prothrombin in the liver appears to be dependent upon the mitochondrial enzyme systems of the liver cell. This was pointed out several years ago by Lasch and co-workers. They have continued their studies on the reciprocal relationship which appears to exist between prothrombin and Factor VII, mediated through the liver (302, 303). The hypothesis is advanced that prothrombin is degraded to thrombin and Factor VII during latent coagulation which is continually in progress as the blood circulates. Factor VII is reconverted to prothrombin as the blood flows through the liver. Barnhart (304) has shown that prothrombin, inactivated by platelet factor 3, AcG, and calcium ion, could be regenerated with liver mitochondria or with cathepsin. The data relating vitamin K₁ to electron transport and coupled oxidative phosphorylation in the respiratory chain in liver cells may be part of this same process (305, 306). Bishydroxycoumarin (Dicumarol) uncouples the phosphorylation reaction. Inactivation of rat liver mitochondria by irradiation also uncouples oxidative phosphorylation. Addition of vitamin K₁ alone or with cytochrome c restores the reaction (307, 308). All of these findings lend support to the hypothesis that vitamin K₁ acts as a coenzyme with the liver mitochondrial enzyme system in electron transport as well as in coupled oxidative phosphorylation. Thyroxin has an action similar to dicumarol in mitochondrial studies. It is of interest that thyroxin-treated rats were more sensitive than normal rats to the prothrombinopenic effects of warfarin (309).

Biochemical studies.—Prothrombin has been prepared in a state of very high purity. Goldstein & Alexander have obtained a prothrombin preparation which is not converted to thrombin in strong citrate solutions. Addition of SPCA causes prompt transformation (224). Diisopropyl-fluorophosphate (DFP), an inhibitor of a number of esterases including thrombin, was found to block the autocatalytic activation of purified prothrombin in citrate (111). Plasma and tissue thromboplastic activators of prothrombin were not interfered with by DFP. It is suggested from these experiments that thrombin or a similar esterase is the responsible "prothrombinase" in citrate activation of prothrombin. Miller (310) has prepared bovine prothrombin which is homogeneous as judged by ultracentrifugal and immunochemical studies. It has a specific activity of 33,000 to 34,000 Iowa units per mg. of tyrosine; the best value attained previously was 29,500 units. The presence of only one mole of N-terminal residues gives indirect support to the contention that the prothrombin is of highest purity. An impurity which Miller removed was a carbohydrase, trans- α -glycosylase (311). Miller & Seegers (312) find that prothrombin contains a polyglucose moiety.

The Carter-Warner hypothesis that sulfhydryl-containing compounds inactivate prothrombin by reducing S—S linkages has received additional support from the work of Koppel *et al.* (313). They believe SH-oxidizing agents such as oxidized glutathione interfere with thrombin formation by acting on —SH groups, probably in AcG. The coagulase-reacting factor (CRF) appears to be prothrombin or one of its derivatives (317). On the

other hand, the thrombocyte agglutinating (TAg) factor of plasma and serum appears to be independent of prothrombin (318).

COMMENT

One characteristic of the current literature is the shift in emphasis of many publications. In the past there has been much preoccupation with the development of a comprehensive hypothesis to include all coagulation reactions. Except as a stimulus to the initiation of new experiments, these hypotheses have been of only limited value; practically all of them have been nullified in one or more respects soon after they were proposed because of the appearance of better data. Today it is realized generally that detailed chemical studies will be required before hypotheses of lasting value can be developed. Such chemical studies are beginning to appear, but many uncontrolled and at present seemingly uncontrollable variables still plague the investigator. It appears premature today to try to answer the following questions regarding each factor. Is it a substrate? Is it an enzyme? Is it a cofactor? With what other procoagulant(s) does it interact? With the many capable workers interested in these problems today, one can only anticipate the appearance of more exacting studies than are now available which may help to resolve these important questions.

LITERATURE CITED

1. Mann, F. D., *Ann. Rev. Physiol.*, **19**, 205-30 (1957)
2. Macfarlane, R. G., *Physiol. Revs.*, **36**, 479-502 (1956)
3. Biggs, R., and Macfarlane, R. G., *Human Blood Coagulation and Its Disorders* (Charles C Thomas, Publisher, Springfield, Ill., 476 pp., 1957)
4. Quick, A. J., *Hemorrhagic Diseases* (Lea & Febiger, Philadelphia, Pa., 451 pp., 1957)
5. Brinkhous, K. M., Ed., *Hemophilia and Hemophiloid Diseases* (University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
6. Brinkhous, K. M., Langdell, R. D., and Wagner, R. H., *Ann. Rev. Med.*, **9**, 159-90 (1958)
7. Schulman, I., and Smith, C. H., *Advances in Pediat.*, **9**, 231-76 (1957)
8. Ratnoff, O. D., *Advances in Internal Med.*, **9**, 107-79 (1958)
9. Hjort, P. F., *Scand. J. Clin. & Lab. Invest.*, **9**, Suppl. 27 (1957)
10. Perlick, E., *Antikoagulantien* (VEB Georg Thieme, Leipzig, Germany, 290 pp., 1957)
11. Scheraga, H. A., and Laskowski, M., *Advances in Protein Chem.*, **12**, 1-131 (1957)
12. Astrup, T., *Lancet*, **II**, 565-68 (1956)
13. Astrup, T., *Blood*, **11**, 781-806 (1956)
14. Martin, G. J., Ed., *Ann. N. Y. Acad. Sci.*, **68** (Art 1), 1-244 (1957)
15. De Nicola, P., *The Thrombelastography* (Charles C Thomas, Publisher, Springfield, Ill., 110 pp., 1957)
16. Morawitz, P., *The Chemistry of Blood Coagulation* (Transl. by Hartmann, R. C., and Guenther, P. F. (Charles C Thomas, Publisher, Springfield, Ill., 194 pp., 1958)

17. Koller, F., *Thrombosis et Diathesis Haemorrhagica*, **1**, 289-350 (1957)
18. Koller, F., *Thrombosis et Diathesis Haemorrhagica*, **1**, 570-608 (1957)
19. Carter, J. R., *Arch. Internal Med.*, **101**, 1178-79 (1958)
20. Wright, I. S., *J. Am. Med. Assoc.*, **163**, 358-59 (1957)
21. Verstraete, M., *Schweiz. med. Wochschr.*, **87**, 1261 (1957)
22. Soulier, J. P., *Rev. franç. études clin. et biol.*, **2**, 894 (1957)
23. Nomenclature of Blood Clotting Factors, *Brit. Med. J.*, **1**, 278 (1957)
24. De Nicola, P., *Il progr. med.*, **13**, 118-24 (1957)
25. *Thrombosis and Embolism*, 1208-9 (Benno Schwabe, Basel, Switzerland, 1316 pp., 1955)
26. Soulier, J. P., *Rev. hématol.*, **11**, 291-93 (1957)
27. Hougie, C., Barrow, E. M., and Graham, J. B., *J. Clin. Invest.*, **36**, 485-96 (1957)
28. Graham, J. B., Barrow, E. M., and Hougie, C., *J. Clin. Invest.*, **36**, 497-503 (1957)
29. Seegers, W. H., Landaburu, R. H., Holburn, R. R., and Tocantins, L. M., *Proc. Soc. Exptl. Biol. Med.*, **95**, 583-85 (1957)
30. Graham, J. B., Collins, D. L., Godwin, I. D., and Brinkhous, K. M., *Proc. Soc. Exptl. Biol. Med.*, **77**, 294-96 (1951)
31. Langdell, R. D., Wagner, R. H., and Brinkhous, K. M., *J. Lab. Clin. Med.*, **41**, 637-47 (1953)
32. Quick, A. J., and Hussey, C. V., *Arch. Internal Med.*, **97**, 524-41 (1956)
33. Nilsson, I. M., Blombäck, M., and von Franken, I., *Acta Med. Scand.*, **159**, 35-57 (1957)
34. Biggs, R., *Lancet*, **II**, 311-14 (1957)
35. Wagner, R. H., Richardson, B. A., and Brinkhous, K. M., *Thrombosis et Diathesis Haemorrhagica*, **1**, 1-8 (1957)
36. Wolf, P., *Brit. J. Haematol.*, **2**, 386-96 (1956)
37. Pitney, W. R., *Brit. J. Haematol.*, **2**, 250-64 (1956)
38. Pool, J. C., in *Proceedings of the 6th International Congress of the International Society of Hematology*, 516 (Grune & Stratton, Inc., New York, N. Y., 930 pp., 1958)
39. Pitney, W. R., *Proc. Roy. Soc. Med.*, **49**, 187-90 (1956)
40. Bounameaux, Y., *Acta Haematol.*, **17**, 355-59 (1957)
41. Miale, J. B., and Garrett, V. R., *Am. J. Clin. Pathol.*, **27**, 701-6 (1957)
42. Miale, J. B., Wilson, M. P., and Garrett, V. R., *Am. J. Clin. Pathol.*, **26**, 969-83 (1956)
43. Miale, J. B., Wilson, M. P., and Garrett, V. R., *Am. J. Clin. Pathol.*, **26**, 984-97 (1956)
44. Shinowara, G. Y., *Am. J. Med. Sci.*, **233**, 528-37 (1957)
45. Johnson, S. A., and Seegers, W. H., in *Hemophilia and Hemophiloid Diseases*, 27-33 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
46. Soulier, J. P., in *Hemophilia and Hemophiloid Diseases*, 18-22 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
47. Sharp, A. A., and Bidwell, E., *Lancet*, **II**, 359-62 (1957)
48. Macfarlane, R. G., Mallam, P. C., Witts, L. J., Bidwell, E., Biggs, R., Fraenkel, G. J., Honey, G. E., and Taylor, K. B., *Lancet*, **II**, 251-59 (1957)
49. Rosenthal, R. L., Dreskin, O. H., and Rosenthal, N., *Proc. Soc. Exptl. Biol. Med.*, **82**, 171-74 (1953)

50. Brinkhous, K. M., in *Proceedings of the 6th International Congress of International Society of Hematology*, 463-67 (Grune and Stratton, New York, N. Y., 930 pp., 1958)
51. Winterstein, A., Marbet, R., and Strässle, R., in *Hemophilia and Hemophiloid Diseases*, 11-17 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
52. Hörder, M. H., *Klin. Wochschr.*, **35**, 775-77 (1957)
53. Egli, H., and Kessler, K., *Deut. med. Wochschr.*, **81**, 875-76 (1956)
54. van Creveld, S., Hoorweg, P. G., Den Ottolander, G. J. H., and Veder, H. A., *Acta Haematol.*, **15**, 1-11 (1956)
55. Shinowara, G. Y., *Bibliotheca Haematol.*, **7**, 350-51 (1958)
56. Blombäck, M., *Arkiv Kemi*, **12**, 387-96 (1958)
57. Blombäck, B., and Bolmbäck, M., *Arkiv Kemi*, **10**, 415-43 (1956)
58. Wagner, R. H., and Thelin, G. M., in *Hemophilia and Hemophiloid Diseases*, 3-10 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
59. Kekwick, R. A., and Wolf, P., *Lancet*, **I**, 647-50 (1957)
60. Seegers, W. H., Landaburu, R. H., and Fenichel, R. L., *Am. J. Physiol.*, **190**, 1-7 (1957)
61. Bidwell, E., *Brit. J. Haematol.*, **1**, 386-89 (1955)
62. Wagner, R. H., Pate, D., and Brinkhous, K. M., *Federation Proc.*, **13**, 445 (1954)
63. Surgenor, D. M., and Steele, B. B., *Thrombosis et Diathesis Haemorrhagica*, **1**, 563 (1957)
64. Lewis, J. H., and Merchant, W., *Federation Proc.*, **17**, 96 (1958)
65. Penick, G. D., and Brinkhous, K. M., *Am. J. Med. Sci.*, **232**, 434-41 (1956)
66. Brinkhous, K. M., Penick, G. D., Langdell, R. D., Wagner, R. H., and Graham, J. B., *Arch. Pathol.*, **61**, 6-10 (1956)
67. Mustard, J. F., *Brit. J. Haematol.*, **3**, 202-14 (1957)
68. Penick, G. D., *Proc. Soc. Exptl. Biol. Med.*, **96**, 277-81 (1957)
69. Rizza, C., and Walker, W., *Nature*, **180**, 143-44 (1957)
70. Fantl, P., and Marr, G., *Australian J. Exptl. Biol. Med. Sci.*, **34**, 433-43 (1956)
71. Gross, J. D., Hartmann, R. C., Graham, J. B., and Taylor, C. B., *Bull. Johns Hopkins Hosp.*, **100**, 223-33 (1957)
72. Wagner, R. H., Langdell, R. D., Richardson, B. A., Farrell, R. A., and Brinkhous, K. M., *Proc. Soc. Exptl. Biol. Med.*, **96**, 152-55 (1957)
73. Langdell, R. D., Wagner, R. H., and Brinkhous, K. M., *Proc. Soc. Exptl. Biol. Med.*, **88**, 212-15 (1955)
74. Blombäck, M., and Nilsson, I. M., *Acta Med. Scand.*, **161**, 1-21 (1958)
75. Brinkhous, K. M., and Penick, G. D., in *Thrombosis and Embolism*, 428-33 (Benno Schwabe, Basel, Switzerland, 1316 pp., 1955)
76. Langdell, R. D., in *Hemophilia and Hemophiloid Diseases*, 189-93 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
77. Graham, J. B., *Am. J. Human Genet.*, **8**, 63-79 (1956)
78. Matter, M., Newcomb, T. F., Melly, A., and Finch, C. A., *Am. J. Med. Sci.*, **232**, 421-33 (1956)
79. Brinkhous, K. M., and Graham, J. B., *Blood*, **9**, 254-57 (1954)
80. Pola, V., and Svojitka, J., *Folia Haematol.*, **75**, 43-51 (1957)
81. Choremis, K. B., Zervos, N., Tseverenis, H., and Apostolopoulou, E., *Helv. Paediat. Acta*, **11**, 305-8 (1956)

82. McGovern, J. J., and Steinberg, A. G., *J. Lab. Clin. Med.*, **51**, 386-97 (1958)
83. Douglas, A. S., and Cook, I. A., *Lancet*, **I**, 616-19 (1957)
84. Wilkinson, J. F., Israels, M. C. G., Nour-Eldin, F., and Turner, R. L., *Brit. Med. J.*, **II**, 1528-29 (1957)
85. Taylor, K., and Biggs, R., *Brit. Med. J.*, **I**, 1494-96 (1957)
86. Margolius, A., and Ratnoff, O. D., *J. Clin. Invest.*, **35**, 1316-23, (1956)
87. Gardikas, C., Katsiroumbas, P., and Kottas, C., *Brit. J. Haematol.*, **3**, 377-78 (1957)
88. Didisheim, P., Ferguson, J. H., and Lewis, J. H., *Arch. Internal Med.*, **101**, 347-54 (1958)
89. Fessey, B. M., and Meynell, M. J., *Brit. Med. J.*, **II**, 391-92 (1957)
90. Klesper, R., and Achenbach, W., *Thrombosis et Diathesis Haemorrhagica*, **1**, 223-33 (1957)
91. Klesper, R., and Achenbach, W., *Klin. Wochschr.*, **35**, 1007-13 (1957)
92. Achenbach, W., and Klesper, R., *Folia Haematol.*, **1**, 251-67 (1957)
93. Thomas, J. W., Black, L., and Perry, W. H., *Can. Med. Assoc. J.*, **77**, 490-92 (1957)
94. Schulman, I., Smith, C. H., Erlandson, M., Fort, E., and Lee, R. E., *Pediatrics*, **18**, 347-61 (1956)
95. Singer, K., and Ramot, B., *Arch. Internal Med.*, **97**, 715-25 (1956)
96. Soulier, J. P., and Alagille, D., *Rev. franç. études clin. et biol.*, **1**, 187-99 (1956)
97. Gross, R., and Mammen, E., *Klin. Wochschr.*, **36**, 112-18 (1958)
98. Brockhaus, J., *Thrombosis et Diathesis Haemorrhagica*, **1**, 529-40 (1957)
99. Nilsson, I. M., Blombäck, M., Jorpes, E., Blombäck, B., and Johansson, S. A., *Acta Med. Scand.*, **159**, 179-88 (1957)
100. Koch, F., Schultze, H. E., Schwick, G., Klees, E., and Kuntze, E., *Z. Kinderheilk.*, **79**, 449-64 (1957)
101. Jürgens, R., Lehmann, W., Wegelius, O., Eriksson, A. W., Hiepler, E., *Thrombosis et Diathesis Haemorrhagica*, **1**, 257-60 (1957)
102. Deutsch, E., *Thrombosis et Diathesis Haemorrhagica*, **1**, 261-63 (1957)
103. Nilsson, I. M., Blombäck, B., Blombäck, M., and Svennerud, S., *Nord. Med.*, **56**, 1654-56 (1956)
104. Biggs, R., and Macfarlane, R. G., *Brit. J. Haematol.*, **4**, 1-27 (1958)
105. McElfresh, A. E., *J. Pediat.*, **51**, 474-82 (1957)
106. Brinkhous, K. M., *Proc. Soc. Exptl. Biol. Med.*, **66**, 117-29 (1947)
107. Bergsagel, D. E., and Hougie, C., *Brit. J. Haematol.*, **2**, 113-29 (1956)
108. Owren, P. A., *Northwest Med.*, **56**, 31-39 (1957)
109. Shafir, E., de Vries, A., and Krejnis, E., *Acta Haematol.*, **16**, 204-13 (1956)
110. Therriault, D. G., Gray, J. L., and Jensen, H., *Proc. Soc. Exptl. Biol. Med.*, **95**, 207-11 (1957)
111. Miller, K. D., and Van Vunakis, H., *J. Biol. Chem.*, **223**, 227-37 (1956)
112. Johnson, S. A., and Seegers, W. H., *Proc. Soc. Exptl. Biol. Med.*, **92**, 597-98 (1956)
113. Shafir, E., and de Vries, A., *J. Clin. Invest.*, **35**, 1183-90 (1956)
114. Graham, J. B., Penick, G. D., and Brinkhous, K. M., *Am. J. Physiol.*, **164**, 710-15 (1951)
115. Douglas, A. S., *Blood*, **11**, 423-29 (1956)
116. Brinkhous, K. M., and Wagner, R. H., *Intern. Congr. Biochem., 4th Cong., Symposia* (In press)
117. Brayton, R. G., and Zucker, M. B., *Proc. Soc. Exptl. Biol. Med.*, **96**, 418-21 (1957)

118. Penick, G. D., Roberts, H. R., Webster, W. P., and Brinkhous, K. M., *Arch. Pathol.* (In press, 1958)
119. Axelrod, S. L., *J. Lab. Clin. Med.*, **48**, 690-701 (1956)
120. Mann, F. D., *J. Lab. Clin. Med.*, **48**, 51-54 (1956)
121. Fonio, A., *Schweiz. med. Wochschr.*, **86**, 1439-41 (1956)
122. Nour-Eldin, F., and Wilkinson, J. F., *Nature*, **180**, 990-91 (1957)
123. Nour-Eldin, F., and Wilkinson, J. F., *Brit. H. Haematol.*, **4**, 38-50 (1958)
124. Huser, H. J., Moor-Jankowski, J. K., Truog, G., and Geiger, M., *Acta Genet. et Statist. Med.*, **8**, 25-50 (1958)
125. Biggs, R., *Brit. J. Haematol.*, **4**, 192-200 (1958)
126. Carroll, R. T., Holburn, R. R., Schwartz, I. R., and Tocantins, L. M., *Clin. Research*, **4**, 5-6 (1956)
127. Graham, J. B., and Barrow, E. M., *J. Exptl. Med.*, **106**, 273-92 (1957)
128. Verstraete, M., *Hemophilia and Hemophiloid Diseases*, 34-50 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
129. Ratnoff, O. D., and Margolius, A., *New Engl. J. Med.*, **256**, 845-46 (1957)
130. Hartmann, J. R., and Diamond, L. K., *Practitioner*, **178**, 179-90 (1957)
131. Harmon, M. C., Zipursky, A., and Lahey, M. E., *J. Diseases Children*, **93**, 375-84 (1957)
132. Verstraete, M., and Vandenbroucke, J., *Am. J. Med.*, **22**, 624-35 (1957)
133. Deutsch, E., *Thrombosis et Diathesis Haemorrhagica*, **1**, 93-113 (1957)
134. Neumark, E., *Acta Haematol.*, **15**, 334-36 (1956)
135. Sharp, A. A., *Nature*, **179**, 632-33 (1957)
136. Wilkinson, J. F., Turner, R. L., and Bottomley, A. C., *Acta Med. Scand.*, **156**, 458-67 (1957)
137. Stefanini, M., and Moschides, E., in *Hemophilia and Hemophiloid Diseases*, 180-84 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
138. Hicks, N. D., and Pitney, W. R., *Brit. J. Haematol.*, **3**, 227-37 (1957)
139. Quick, A. J., in *Hemophilia and Hemophiloid Diseases*, 169-73 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
140. Colebatch, J. H., and Wilson, B. M., *Med. J. of Australia*, **1**, 226-29 (1956)
141. Cooper, T., and Owen, C. A., *Med. Clin. N. Am.*, **40**, 1173-85 (1956)
142. Koller, F., *Deut. med. Wochschr.*, **81**, 516-24 (1956)
143. Larrieu, M. J., *Rev. prat. (Paris)*, **6**, 2307-22 (1956)
144. van Creveld, S., *Pediat. intern.*, **1**, 1-27 (1956)
145. Holzknecht, F., *Med. Klin. (Munich)*, **51**, 1559-61 (1956)
146. Hule, V., *Vnitřní lékařství*, **2**, 564-66 (1956)
147. Spaet, T. H., *Am. Practitioner and Dig. Treatment*, **7**, 403-7 (1956)
148. Jolly, W. P., *J. Iowa State Med. Soc.*, **46**, 181-86 (1956)
149. Bernard, J., and Beaumont, J. L., *Rev. prat. (Paris)*, **6**, 2323-26 (1956)
150. Comer, F., *Arch. belges méd. sociale, hyg., méd. travail et méd. légale*, **14**, 437-47 (1956)
151. Nour-Eldin, F., and Wilkinson, J. F., *Brit. J. Haematol.*, **2**, 433-38 (1956)
152. Nour-Eldin, F., and Wilkinson, J. F., *Nature*, **179**, 532-33 (1957)
153. Nour-Eldin, F., and Wilkinson, J. F., *J. Physiol. (London)*, **136**, 324-32 (1957)
154. Richards, M. D., and Spaet, T. H., *Blood*, **11**, 473-83 (1956)
155. Windorfer, A., Schultze, H. E., and Schwick, G., *Blut*, **2**, 217-29 (1956)
156. Peñalver, J. A., Holburn, R. R., Carroll, R. T., Baird, H., and Tocantins, L. M.,

- in *Hemophilia and Hemophiloid Diseases*, 57-59 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
157. Frick, P. G., *Blood*, **10**, 691-706 (1955)
 158. Peñalver, J. A., Holburn, R. R., Carroll, R. T., and Tocantins, L. M., *Thrombosis et Diathesis Haemorrhagica*, **1**, 353-58 (1957)
 159. Rosenthal, M. C., in *Hemophilia and Hemophiloid Diseases*, 51-56 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
 160. Lewis, J. H., Ferguson, J. H., and Arends, T., *Blood*, **11**, 846-55 (1956)
 161. Bergna, L. J., and Pavlovsky, A., *Acta Haematol.*, **16**, 247-54 (1956)
 162. Verstraete, M., and Vandenbroucke, J., *J. Lab. Clin. Med.*, **48**, 673-89 (1956)
 163. Nilsson, I. M., Skanse, B., and Gydell, K., *Acta Haematol.*, **19**, 40-50 (1958)
 164. Nussey, A. M., and Dawson, D. W., *Brit. Med. J.*, **II**, 1077-79 (1957)
 165. Speer, R. J., Hill, J. M., Maloney, M., Roberts, A., and Prager, M. D., *Am. J. Clin. Pathol.*, **26**, 477-86 (1956)
 166. Rodman, N. F., Barrow, E. M., and Graham, J. B., *Am. J. Clin. Pathol.*, **29**, 525-38 (1958)
 167. Wartelle, O., *Rev. hématol.*, **11**, 414-25 (1956)
 168. Biggs, R., Sharp, A. A., Margolis, J., Hardisty, R. M., Stewart, J., and Davidson, W. M., *Brit. J. Haematol.*, **4**, 177-91 (1958)
 169. Sjölin, K. E., *Danish Med. Bull.*, **3**, 222-23 (1956)
 170. Sjölin, K. E., *Proc. Soc. Exptl. Biol. Med.*, **94**, 818-20 (1957)
 171. Seaman, A. J., and Karlsen, K. M., *Clin. Research*, **6**, 79-80 (1958)
 172. Spaet, T. H., Ratnoff, O. D., Graham, J. B., Schulman, J., and Rosenthal, R. L., *J. Lab. Clin. Med.*, **52**, 634-40 (1958)
 173. Seegers, W. H., and Johnson, S. A., *Am. J. Physiol.*, **184**, 259-64 (1956)
 174. Freudenberg, E., and Oeri, J., *Ann. Paediat.*, **188**, 89-105 (1957)
 175. Aggeler, P. M., in *Hemophilia and Hemophiloid Diseases*, 111-15 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
 176. Barkhan, P., *Brit. J. Haematol.*, **3**, 215-19 (1957)
 177. Haanen, C. A. M., *Acta Haematol.*, **16**, 363-75 (1956)
 178. McElfresh, A. E., and Özge, A., *J. Lab. Clin. Med.*, **49**, 753-55 (1957)
 179. Naeye, R. L., *Proc. Soc. Exptl. Biol. Med.*, **91**, 101-4 (1956)
 180. Naeye, R. L., *Proc. Soc. Exptl. Biol. Med.*, **94**, 623-27 (1957)
 181. Lewis, J. H., Ferguson, J. H., Spaugh, E., Fresh, J. W., and Zucker, M. B., *Blood*, **12**, 84-89 (1957)
 182. Johnson, S. A., and Seegers, W. H., *Circulation Research*, **4**, 182-86 (1956)
 183. Verstraete, M., and Vandenbroucke, J., *Arch. intern. pharmacodynamie*, **109**, 439-45 (1957)
 184. Sise, H. S., Adamis, D., and Kimball, D., *J. Lab. Clin. Med.*, **49**, 69-83 (1957)
 185. McElfresh, A. E., Sharpsteen, J. R., and Akabane, T., *Pediatrics*, **17**, 870-76 (1956)
 186. Fresh, J. W., Ferguson, J. H., Stamey, C., Morgan, F. M., and Lewis, J. H., *Pediatrics*, **19**, 241-51 (1957)
 187. Fresh, J. W., Ferguson, J. H., and Lewis, J. H., *Obstet. and Gynecol.*, **7**, 117-27 (1956)
 188. Cowling, D. C., *J. Clin. Pathol.*, **9**, 347-50 (1956)
 189. Firkin, B. G., *Med. J. of Australia*, **I**, 557-58 (1958)
 190. Aggeler, P. M., Hoag, M. S., and Wallerstein, R. O., *J. Am. Med. Women's Assoc.*, **12**, 280-83 (1957)

191. Birk, G., *Klin. Wochschr.*, **36**, 240 (1958)
192. Rosin, S., Moor-Jankowski, J. K., Schneeberger, M., *Acta Genet. et Statist. Med.*, **8**, 1-24 (1958)
193. Moor-Jankowski, J. K., Truog, G., and Huser, H. J., *Acta Genet. et Statist. Med.*, **7**, 597-780 (1957)
194. Hule, V., and Nešpůrková, M., *Ann. Paediat.*, **186**, 175-81 (1956)
195. Hardisty, R. M., *Brit. Med. J.*, **1**, 1039-40 (1957)
196. Nour-Eldin, F., and Wilkinson, J. F., *Clin. Sci.*, **17**, 303-7 (1958)
197. Heni, F., and Krauss, I., *Deut. med. Wochschr.*, **81**, 1603-5 (1956)
198. Brafeld, A. J., and Case, J., *Lancet*, **II**, 867-69 (1956)
199. Brinkhous, K. M., in *Hemophilia and Hemophiloid Diseases*, 132 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
200. Fantl, P., Sawers, R. J., and Marr, A. G., *Australasian Ann. Med.*, **5**, 163-76 (1956)
201. Fantl, P., and Sawers, R. J., *Nature*, **177**, 1233-34 (1956)
202. Lewis, J. H., and Didisheim, P., *Proc. Soc. Exptl. Biol. Med.*, **93**, 429-32 (1956)
203. Fantl, P., in *Hemophilia and Hemophiloid Diseases*, 79-92 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
204. Graham, J. B., in *Hemophilia and Hemophiloid Diseases*, 137-62 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
205. Larrieu, M. J., Caen, J., Grenet, P., Cayla, J., and Bernard, J., *Le Sang*, **27**, 117-30 (1956)
206. Lewis, J. H., Ferguson, J. H., Fresh, J. W., and Zucker, M. B., *J. Lab. Clin. Med.*, **49**, 211-32 (1957)
207. O'Brien, J. R., *Brit. J. Haematol.*, **4**, 210-19 (1958)
208. Hougie, C., *J. Lab. Clin. Med.*, **50**, 61-68 (1957)
209. Turpini, R., *Experientia*, **12**, 220-22 (1956)
210. Katz, R., and Ducci, H., *Thrombosis et Diathesis Haemorrhagica*, **1**, 413-17 (1957)
211. Stormorken, H., *Scand. J. Clin. & Lab. Invest.*, **9**, 273-76 (1957)
212. Seaman, A. J., and Owren, P. A., *J. Clin. Invest.*, **35**, 145-49 (1956)
213. Stormorken, H., *Acta Physiol. Scand.*, **39**, 121-36 (1957)
214. Leikin, S., and Bessman, S. P., *Blood*, **11**, 916-23 (1956)
215. Zucker, M. B., and Borrelli, J., *J. Appl. Physiol.*, **12**, 453-60 (1958)
216. Cox, F. M., Lanchantin, G. F., and Ware, A. G., *J. Clin. Invest.*, **35**, 106-13 (1956)
217. Johnson, J. F., *Proc. Soc. Exptl. Biol. Med.*, **94**, 92-94 (1957)
218. Carter, J. R., and Warner, E. D., *J. Clin. Invest.*, **37**, 70-86 (1958)
219. Fajardo, L. F., and Silvert, D., *Am. J. Obstet. Gynecol.*, **74**, 909-14 (1957)
220. Seegers, W. H., Alkjaersig, N., and Johnson, S. A., *Am. J. Clin. Pathol.*, **25**, 983-87 (1955)
221. Ferguson, J. H., Johnston, C. L., and Howell, D. A., *Proc. Soc. Exptl. Biol. Med.*, **95**, 567-70 (1957)
222. Ferguson, J. H., Johnston, C. L., and Howell, D. A., *Blood*, **13**, 382-97 (1958)
223. Hougie, C., Barrow, E. M., and Graham, J. B., *Bibliotheca Haematol.*, **7**, 336-40 (1958)
224. Goldstein, R., and Alexander, B., in *Hemophilia and Hemophiloid Diseases*, 93-110 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)

225. Hougie, C., *Proc. Soc. Exptl. Biol. Med.*, **93**, 570-73 (1956)
226. Barnett, C. P., *Arch. Internal Med.*, **99**, 280-84 (1957)
227. Bachmann, F., Duckert, F., Flückiger, P., Hitzig, W. H., and Koller, F., *Thrombosis et Diathesis Haemorrhagica*, **1**, 87-92 (1957)
228. Telfer, T. P., Denson, K. W., and Wright, D., *Brit. J. Haematol.*, **2**, 308-16 (1956)
229. Bachmann, F., Duckert, F., Geiger, M., Baer, P., and Koller, F., *Thrombosis et Diathesis Haemorrhagica*, **1**, 169-94 (1957)
230. Gonyea, L. M., Krivit, W., *J. Lab. Clin. Med.*, **51**, 398-409 (1958)
231. Hörder, M. H., *Acta Haematol.*, **19**, 30-39 (1958)
232. Oehme, J., Schwick, G., and Schultze, H. E., *Klin. Wochschr.*, **36**, 521-24 (1958)
233. Sise, H. S., Lavelle, S. M., and Becker, R., *Proc. Soc. Exptl. Biol. Med.*, **96**, 662-64 (1957)
234. Bachmann, F., Duckert, F., and Koller, F., *Thrombosis et Diathesis Haemorrhagica*, **2**, 24-38 (1958)
235. Hörder, M. H., *Thrombosis et Diathesis Haemorrhagica*, **2**, 171-78 (1958)
236. Bachmann, F., Duckert, F., Flückiger, P., and Hitzig, W. H., *Schweiz. med. Wochschr.*, **87**, 1221-23 (1957)
237. Gonyea, L. M., Hjort, P., and Owren, P. A., *J. Lab. Clin. Med.*, **48**, 624-33 (1956)
238. Burmeister, A., *Z. Kinderheilk.*, **81**, 88-101 (1958)
239. van Creveld, S., Veder, H. A., Blans, M. M., *Le Sang*, **28**, 23-34 (1957)
240. Jürgens, J., *Acta Haematol.*, **16**, 181-98 (1956)
241. Koch, F., Schultze, H. E., Schwick, G., and Beller, F. K., *Z. Kinderheilk.*, **76**, 208-33 (1955)
242. Hicks, N. D., *Med. J. of Australia*, **II**, 331-35 (1955)
243. Owren, P. A., *Am. J. Med.*, **14**, 201-15 (1953)
244. Jenkins, J. S., *J. Clin. Pathol.*, **7**, 29-31 (1954)
245. Ackroyd, J. F., *Brit. J. Haematol.*, **2**, 397-411 (1956)
246. Pitney, W. R., *Australasian Ann. Med.*, **7**, 15-26 (1958)
247. van Creveld, S., Veder, H. A., and Blans, M. M., *Ann. Paediat.*, **187**, 373-77 (1956)
248. van Creveld, S., and Veder, H. A., *Ann. Paediat.*, **190**, 316-20 (1958)
249. Dische, F. E., *Brit. J. Haematol.*, **4**, 201-9 (1958)
250. Choremis, C., Padiatellis, C., Tseveren, I., and Hadjidimitriou, E., *Helv. Paediat. Acta*, **11**, 301-4 (1956)
251. Zbinden, J., and Leupold, R., *Schweiz. med. Wochschr.*, **87**, 1478-80 (1957)
252. Frick, P. G., *Acta Haematol.*, **19**, 20-29 (1958)
253. Biggs, R., *Brit. J. Haematol.*, **2**, 412-20 (1956)
254. Greig, H. B., and Tattersall, J. C., *Brit. J. Haematol.*, **2**, 421-29 (1956)
255. Chirico, A. M., and McElfresh, A. E., *Blood*, **12**, 933-41 (1957)
256. Ratnoff, O. D., and Colopy, J. E., *J. Clin. Invest.*, **34**, 602-13 (1955)
257. Johnston, C. L., and Ferguson, J. H. (Personal communication)
258. Ratnoff, O. D., and Rosenblum, J. M., *J. Lab. Clin. Med.*, **50**, 941-42 (1957)
259. Margolis, J., *Nature*, **180**, 1464-65 (1957)
260. Larrieu, M. J., Soulier, J. P., and Culot, Y., *Le Sang*, **28**, 152-59 (1957)
261. Greig, H. B. W., Falcke, H. C., Simon, M., and Cohen, H., *Arch. Disease Childhood*, **31**, 293-97 (1956)
262. Ramot, B., Singer, K., Heller, P., and Zimmerman, H. J., *Blood*, **11**, 745-52 (1956)

263. Sjølin, K. E., *Thrombosis et Diathesis Haemorrhagica*, **1**, 153-57 (1957)
264. Frick, P. G., and Hagen, P. S., *J. Lab. Clin. Med.*, **47**, 592-601 (1956)
265. Margolius, A., and Ratnoff, O. D., *Blood*, **11**, 565-69 (1956)
266. Margolius, J., *J. Physiol. (London)*, **137**, 95-109 (1957)
267. Jim, R. T. S., and Goldfein, S., *Am. J. Med.*, **23**, 824-31 (1957)
268. Ratnoff, O. D., and Margolius, A., in *Hemophilia and Hemophiloid Diseases*, 124-29 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
269. Quick, A. J., *Bibliotheca Haematol.*, **7**, 341-45 (1958)
270. Soulier, J. P., and Larrieu, M. J., *Thrombosis et Diathesis Haemorrhagica*, **2**, 1-23 (1958)
271. Margolis, J., *Nature*, **181**, 635-36 (1958)
272. Margolis, J., *Nature*, **178**, 805-6 (1956)
273. Chevallier, P., Fiehrer, A., and Samama, M., *Le Sang*, **27**, 950-53 (1956)
274. Gouttas, A., Tsevenis, H., Priovolos, J., Fessas, P., and Mandalaki, T., *Le Sang*, **28**, 141-51 (1957)
275. Caen, J., and Bernard, J., *Le Sang*, **27**, 249-57 (1956)
276. Campbell, E. W., and Mednicoff, I. B., *Clin. Research*, **4**, 6 (1956)
277. Stegelske, R. F., Gores, R. J., Hurn, M. M., and Owen, C. A., *Oral Surg., Oral Med. Oral Pathol.*, **10**, 225-29 (1957)
278. Henry, E. I., Rosenthal, R. L., and Hoffman, I., *J. Am. Med. Assoc.*, **162**, 727-29 (1956)
279. Campbell, E. W., Mednicoff, I. B., and Dameshek, W., *Arch. Internal Med.*, **100**, 232-40 (1957)
280. Rosenthal, R. L., in *Hemophilia and Hemophiloid Diseases*, 116-23 (Brinkhous, K. M., Ed., University of North Carolina Press, Chapel Hill, N. C., 265 pp., 1957)
281. Rosenthal, R. L., and Gendelman, E., *J. Lab. Clin. Med.*, **45**, 123-29 (1955)
282. Ramot, B., Angelopoulos, B., and Singer, K., *Arch. Internal Med.*, **95**, 705-12 (1955)
283. Lisker, R., Josephson, A. M., Werbin, G., Shapiro, C. M., and Rosengvaig, S., *A.M.A. Arch. Internal Med.*, **100**, 474-77 (1957)
284. Chevallier, P., Samama, M., Bilski-Pasquier, G., and Fiehrer, A., *Le Sang*, **29**, 65-73 (1958)
285. Josephson, A. M., and Lisker, R., *J. Clin. Invest.*, **37**, 148-52 (1958)
286. Dreskin, O. H., *J. Lab. Clin. Med.*, **51**, 312-16 (1958)
287. Burstein, M., *Rev. hématol.*, **12**, 500-6 (1957)
288. Glueck, H. I., *Bibliotheca Haematol.*, **7**, 358-62 (1958)
289. Arscott, P. M., Koppel, J. L., and Olwin, J. H., *J. Lab. Clin. Med.*, **51**, 805-13 (1958)
290. Seegers, W. H., Levine, W. G., and Shepard, R. S., *Can. J. Biochem. and Physiol.*, **36**, 603-11 (1958)
291. Landaburu, R. H., and Seegers, W. H., *Am. J. Physiol.*, **193**, 169-80 (1958)
292. Gollub, S., Black, J., and Ulin, A. W., *J. Lab. Clin. Med.*, **50**, 326-29 (1957)
293. Neilson, D. B., and Briggs, E. M. G., *Scot. Med. J.*, **2**, 284-87 (1957)
294. Orekhova, A. A., *Biull. eksptl. biol. med.* (in English transl.), **44**, 966-69 (1957)
295. Dyggve, H., *Acta Paediat.*, **47**, 251-59 (1958)
296. Brinkhous, K. M., Smith, H. P., and Warner, E. D., *Am. J. Med. Sci.*, **193**, 475-80 (1937)
297. Stormorken, H., *Acta Physiol. Scand.*, **41**, 101-17 (1957)
298. Brinkhous, K. M., and Walker, S. A., *Am. J. Physiol.*, **132**, 666-69 (1941)

- 299. von Kaulla, K. N., and Pratt, E. B., *Am. J. Physiol.*, **187**, 89-93 (1956)
- 300. Fisher, L. M., Millar, G. J., and Jaques, L. B., *Can. J. Biochem. and Physiol.*, **34**, 1039-51 (1956)
- 301. Douglas, A. S., *J. Clin. Pathol.*, **11**, 261-64 (1958)
- 302. Lasch, H. G., Mechelke, K., and Nusser, E., *Deut. Arch. klin. Med.*, **204**, 1-21 (1957)
- 303. Lasch, H. G., Pfisterer, I., and Schimpf, K., *Acta Haematol.*, **17**, 280-87 (1957)
- 304. Barnhart, M. I., *Am. J. Physiol.*, **189**, 527-32 (1957)
- 305. Chance, B., and Williams, G. R., *Advances in Enzymol.*, **17**, 65-134 (1956)
- 306. Cooper, C., and Lehninger, A. L., *J. Biol. Chem.*, **219**, 489-529 (1956)
- 307. Beyer, R. E., *Biochim. et Biophys. Acta*, **28**, 663-64 (1958)
- 308. Dallam, R. D., and Anderson, W. W., *Biochim. et Biophys. Acta*, **25**, 439 (1957)
- 309. Lowenthal, J., and Fisher, L. M., *Experientia*, **13**, 253-54 (1957)
- 310. Miller, K. D., *J. Biol. Chem.*, **231**, 987-95 (1958)
- 311. Miller, K. D., and Copeland, W. H., *Biochim. et Biophys. Acta*, **22**, 193-94 (1956)
- 312. Miller, K. D., and Seegers, W. H., *Arch. Biochem. Biophys.*, **60**, 398-401 (1956)
- 313. Koppel, J. L., Mueller, D., and Olwin, J. H., *Am. J. Physiol.*, **187**, 113-21 (1956)
- 314. Aballi, A. I., Lopez Banus, V., de Lamerens, S., and Rosengvaig, S., *J. Diseases Children*, **94**, 589-600 (1957)
- 315. Flückiger, P., Hitzig, W. H., Bachmann, F., and Duckert, F., *Helv. Paediat. Acta*, **12**, 260-70 (1957)
- 316. Sjölin, K. E., *Acta Genet. et Statist. Med.*, **7**, 541-48 (1957)
- 317. Tager, M., *J. Exptl. Med.*, **104**, 675-86 (1956)
- 318. Brinkhous, K. M., LeRoy, E. C., Cornell, W. P., Brown, R. C., Hazlehurst, J. L., and Vennart, G. P., *Proc. Soc. Exptl. Biol. Med.*, **98**, 379-83 (1958)

BIOPHYSICAL ASPECTS OF CONDUCTION AND TRANSMISSION IN THE NERVOUS SYSTEM^{1,2}

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A review in this area should consider four anatomical levels: (a) receptor-neural junctions; (b) axons; (c) synapses, cell bodies, and functioning cell groups; and (d) neuroeffector junctions. At each level, structural, chemical, and electrical parameters should be dealt with. Receptors and effectors too should be included; their resemblance to, and intimate interaction with, neural tissue is such that understanding one abets understanding the other. Intercellular trophic phenomena influence conducting substrata and also merit attention. Indeed, suitable topics are legion. Among them might be the electrophysiology of single cardiac cells (2) and of the heart as a whole (12), drug actions on bioelectric phenomena (7), neural function of acetylcholine (11), posttetanic potentiation (15), brain tissue lipides (16), electrical properties of tissues and cell suspensions (21), and chemical mechanisms of cellular excitation (25). This review will, however, center on the levels listed.

RECEPTONEURAL JUNCTION

Structure.—The major source of information is electron microscopy. A controversial point, which is also relevant for other junctions, immediately arises. It is noted here for later recall: at cochlear and taste bud junctions synaptic vesicles seem to be mainly postjunctional [Smith & Dempsey (237); De Lorenzo (79)]. The likelihood of efferents to the junctions complicates this conclusion, but that these vesicles are truly postjunctional must be considered before casually assigning a chemical mediator role to them (section on Synapses and Cell Bodies). [The term "vesicle" is used here with the same reservations as for "pore" (section on Axon: structure and composition). No evidence exists that these "vesicles" are vesicles in any dictionary sense.]

In one of the few experiments coupling electron microscopy with function, rod and cone presynaptic vesicles changed size in animals in the dark [DeRobertis & Franchi (81)]. Also interesting is the large number of mitochondria at junctions and their preference for just subsurface positioning in cochlear hair cells [Smith & Dempsey (237)], Pacinian corpuscle fibers [Pease & Quilliam (200)], squid and lobster axons [Geren & Schmitt (114),

¹ The survey of the literature pertaining to this review was concluded in June, 1958.

² The following abbreviations and symbols will be used: ACh (acetylcholine); []_i and []_o (concentration inside and outside); GABA (γ -aminobutyric acid).

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Tobias (269)]. The comment that such positioning puts mitochondrial energy converters where they are most needed becomes a cliché.

Other findings which will one day fit a molecular level description of junctional activity follow. Nerve ends in corneal epithelial cells are separated from cytoplasm only by an infolded membrane [Whitear (282)]. Olfactory rod membrane invaginations connect with cytoplasmic vesicles [De Lorenzo (78)], recalling Bennett's (42) suggestion that invaginating cell membrane may subserve transsurface transport. The Pacinian corpuscle axon loses myelin before reaching the core, and Schwann sheath soon after; the terminal is naked. Cochlear hair cells show subsurface anastomosing vesicles often juxtaposed with mitochondria; nerve endings cover the hair cell base without interruption; the junction is two osmophilic layers separated by a cleft, with many mitochondria close to the interface on the hair cell side. The nerve-ending mitochondria are farther from the junction, with many vesicles interposed [Smith & Dempsey (237)]. Utricle structure is seen in work of Smith (236), cristae ampullaris in that of Wersäll (281). Insect eye microstructure is described by Goldsmith & Philpott (118) and by Wolken *et al.* (284), that of the *Limulus* eye by Miller (186).

Chemical properties.—One seeks chemical bases for producing molecular structural changes which somehow would develop the generator potential. There has been little systematic progress.

Perhaps the most interesting recent finding is that light changes conductance in rhodopsin solutions, more so in the presence of rod lipid [Hara (125)]. Light might produce similar changes in a semiconductorlike sandwich of rhodopsin layered with lipid in membranous rod discs, and such a change in resistance may be related to the establishment of the generator potential.

Endopeptidases, released by trauma, may excite superficial fibers to produce pruritus [Shelley & Arthur (230)]. Veratrum alkaloids, thought to act on the receptors *per se*, stimulate and desensitize visceral stretch organs [Paintal (196)]; natural stimuli terminate such impulses. Calcium reduces and potassium enhances the alkaloid effect. Acetylcholine may stimulate growing nerve terminals, but one recalls that although ACh excites carotid receptors, still ACh blockage does not prevent response to pressure. Thus, even if ACh can excite, it may not be important in the normal chain of events [Diamond (83)].

Desensitization following chemical excitation is well-known. Understanding it would probably give great insight into excitation and recovery processes. Because little progress has been made [Katz & Thesleff (145)] since Paton's (199) discussion in *Annual Review of Physiology* for 1958, the subject will not be dealt with here.

Neither cochlear nor auditory cortex responses are changed by very low blood sugar, but both are sensitive to hypotension [Fernández & Brenman (99)], thus correlating with surviving brain function at low sugar levels if perfusion is rapid [Geiger (6)]. Acoustic fatigue, dihydrostreptomycin, or quinine affect protein synthesis [Mizukoshi *et al.* (187)]. Organ of Corti sensory elements contain little polysaccharide [Bélanger (39)].

Sodium-L-glutamate in high doses, and, to lesser extent, aspartate, rather specifically damage retinal neurons. This may relate to interference with potassium regulation or with synthesis of high-energy phosphates, but the metabolic lesions are not really known [Lucas & Newhouse (175)]. For extensive studies of chemical effects on retinal physiology see Noell (195). Acetylcholine esterase is found at synapses in developing chick retina [Shen *et al.* (231)], and in the organ of Corti [Churchill *et al.* (63)]. Sjöstrand (234) refers to a higher concentration of aldolase, cytochrome oxidase, cholinesterase, and adenosinetriphosphatase in the inner than in the outer segments of the rods.

Electrophysiology.—Because of speed, sensitivity, and relatively high spatial resolution, electrophysiological techniques continue to be productive.

Loewenstein and co-workers (170, 171, 172) report greatly interesting work on Pacinian corpuscle generator potential and propagated responses. Removal of outer laminae, parts of the core, or terminal axon pieces does not prevent or greatly change function. Axon degeneration eliminates response; local compression of the first node eliminates only the regenerative activity. It seems clear that the generator potential is produced by non-myelinated endings, and regenerative activity at the first intracorporecular node. One wonders what the function may be of the elaborate (206) but in some sense dispensable corpuscle. Changing the sodium level in corpuscle perfusate indicates the receptor potential is related to ions moving down gradients, with sodium carrying most of the charge [Gray & Inman (120)].

That this receptor, as also frog touch receptors, can be changed from rapidly to slowly adapting by stretch is greatly provocative; an hypothesis is based on the notion that

... depolarization and hence generation of impulses occur by an unfolding of the membrane.—Whether a permanent or a transient depolarization of the nerve ending results depends on whether the membrane is held permanently or temporarily expanded [Loewenstein (169)].

Another possibility should be considered. Stretch tends to align macromolecules; and alignment of chain molecules, as in a gel, increases the probability of cross-bonding, thus favoring aggregation into microcrystalline areas and leaving other regions relatively emptier. Generator potential decay might be a function of such microorganization, slow adaptation reflecting stabilization in the pseudodisperse, more excitable state. It would be greatly interesting if one could combine stretch and electrophysiological measurements with quantitative birefringence observations. Technical difficulties might be formidable, but such studies, if feasible, could clarify at a macromolecular level the qualitative differences, if any exist, between the type of structure supporting graded potentials and that underlying regenerative potentials. A change in macromolecular organization associated with impulse conduction also might show up in stretch-oriented tissue but could be lost against a more random background. Stretch reverses the sign of the light-scattering change with activity in *Carcinus* nerve [Bryant & Tobias (50)]; therefore changes in birefringence should be sought in stretched whole nerve. In this context, the suggestion that the naked terminal is somehow

specialized because antidromic impulses do not invade it is interesting [Diamond (84)]. A method for measuring Pacinian corpuscle compression is described by Hubbard (133). Other work on mechanoreceptors is that of Douglas & Ritchie (85), Ito (139), Iggo (137), Cooper & Daniel (67).

Helpful summaries *in re* audition are those of Davis (3, 73) and Whitfield (27). Although great advances have been made in the physics and physiology of audition and its central electrophysiology and organization, the mechanism by means of which hair cell distortion generates a slow potential is unknown. This is a fundamental matter and may partake of underlying events similar to those in changing rhodopsin conductance. In this case a semiconductorlike cell surface may have its resistance changed by mechanical distortion rather than photochemically. However, one may imagine that mechanoreceptors, as the Pacinian corpuscle above, are more directly accessible to investigation of the structure-activity relation. It might also be rewarding to study vestibular cells. Here activity can be increased above resting background by flowing the fluid in one direction, and decreased below it by opposite flow [Lowenstein (173)]. First, one might find whether such changes correlate with de- or hyperpolarization. Second, because of slow adaptation, activity can be increased or decreased for relatively long periods. Thus, perhaps with the additional help of inhibitors such as dinitrophenol (DNP), consequences of greater or lesser activity might be made to accumulate to a level of more ready detectability.

Attempts to locate the retinal generator suffer from electrode positioning uncertainty. Svaetichin (257) [on multiple cones, see also Lyall (178)] concluded that cones which react to light by variable hyperpolarization alone, and those which react by hyper- or depolarization depending on wavelength, can both excite neurons. Drawing on electron microscopic findings, it was argued that hyperpolarizing cones act on the soma like an anode to produce outward exciting current through the initial segment, whereas depolarizing cones contact dendrites of somata farther down. Motokawa *et al.* (188) find that some elements respond with hyper- or depolarization depending on wavelength, but when slow potential and spikes are recorded by a single electrode, hyperpolarization is seen to result in cessation of activity. Tomita (270) concludes that responses like those obtained by Svaetichin probably arise extracellularly, proximal to the receptors. *Limulus* eye studies agree essentially with earlier work; eccentric cell depolarization is thought to drive current to initiate impulses [Tomita (271)]. No further work has appeared on the origin of maintained retinal activity in the dark [Kuffler *et al.* (159)]. This is also found in the completely unopened eye by intracranial optic nerve recording; raising intraocular pressure stops it [Bornschein (45)].

Bullock & Fox (51), reasoning from the anatomy of the pit-viper infrared organ, speculate on the microlocale where background discharge may arise as a result of occasional summation of randomly occurring, subthreshold, graded activity; axon terminals may act as dendrites, not propagating responses but modifying impulse initiation by small changes in potential gradient along the neuronal surface.

AXON

Structure and composition.—Well-rewarded effort has been expended on myelin structure: reasonable correspondence between electron microscopy and x-ray diffraction establishes the repeating unit size and older ideas of structure more firmly, and supports the validity of certain types of electron microscopy [Fernández-Morán & Finean (98)].

Distribution of cholesterol, phospholipide, protein, etc. in myelin remains uncertain. The more osmophilic layers may contain most protein and be mechanically more stable; less dense interspaces may contain more lipid [Robertson (216)]. Myelination probably raises threshold for mechanical excitation (155). Little is known about myelin water, but changes induced in x-ray diffraction pattern by hyper- or hypotonic solutions and nuclear magnetic resonance studies may yield information in this respect [Finean and Millington (100, 102); Fernández-Morán, in (19)]. Myelin lipoprotein from different sources may be different according to Finean *et al.* (101). In a recent book by Engström & Finean (5), Finean summarizes his views about molecular level myelin structure; a basic building block would contain a cerebroside molecule associated with complexes of phospholipide hydrogen bonded to cholesterol [see also Tobias (269)]. Localization of sulfhydryl groups in myelin is not yet successful according to Finean. No attempts to correlate x-ray data with activity since the experiments of Handovsky (124) and of Boehm (44) are known to the writer.

Luse (176) reports that axons in brain are not myelinated by repeated spiral wrapping of the Schwann cell membrane with the same number of layers on all sides, as in nerve; rather, glial membranes are affixed with variable numbers of lamellae in different parts of the sheath, and with numerous attachments instead of one mesaxon. Rabbit myelin glyco- and phosphosphingolipids change until adult age [Edgar (91)]. Rat brain and nerve phosphatides are presumably formed *in situ* by pathways like those for phosphatide synthesis in chicken liver. In degenerating nerve, phosphatide increase is associated with rising Schwann cell number [Rossiter *et al.* (220)].

Uzman & Nogueira-Graf (275) beautifully clarify nodal structure. Nelson (193), Luxoro (177), and Robertson (218, 219) describe nodes and incisures. One must see the Uzman pictures to understand the structure. Suffice it to say, conventional myelin is completely interrupted at the node, but whether Schwann cells fuse or only closely approximate is not certain. Squid and lobster axons show variable numbers of double layered surface lamellae each about as thick as myelin lamellae [Geren & Schmitt (114); Tobias (269)]. These may connect at places but three-dimensional organization is not yet clear. Vesiculated *Helix* axons are separated from each other by only one pair of surface layers about 60 Å thick [Schlote (221)]. The "axolemma" of lobster axons may show occasional regions of lesser density [Tobias (269)] as also described for insect axon "sheaths" by Fernández-Morán (19), who refers to "pores". This plausible and comforting term "pore" is not yet warranted for these structures even though it is concluded on the basis of labelled water exchange that there are transsurface aqueous channels of effective radius *ca.* 16 Å in lobster and 11 Å in squid axons [Schmitt (223); Nevis

(194)]. The finding by electron microscopy that material inside the annulus, i.e., the "pore", may be denser than that outside it is pertinent [Kautz & De Marsh (146)]. In osmicated tissue a "pore" could simply be a region of lesser osmium affinity. Also, though "pores" have often been described for nuclear envelopes, there are no other instances known to the writer when they have been described electron-microscopically for plasma membranes.

Whatever the final evaluation, considerations of ion transport must take notice of such data. Whether Schwann cells fuse across nodes or not; whether there are transverse passages connecting invertebrate surface lamellae; that there are multiple, not just one, surface layers all along invertebrate axons; where the water really is in myelin; and whether there are pores or not are all important matters for any hypothesis *in re* ion movements, as well as for movement of organic substances across the surface.

Axoplasm continues to show fine filaments beaded with an axial-pseudo-periodicity in squid [Maxfield (180)], lobster [Tobias (269)], *Carcinus maenas* [Tobias (unpublished)] and *Odonta* [Fernández-Moran in (19)]; no functional role has been established. Localized changes in ionic strength or pH with impulses could modify aggregation or diameter [Schmitt (223)]. Such ideas are based on work of Maxfield & Hartley (181, 182) on physicochemical characteristics of axon protein. Localized, nonelectrical, physical changes in active axons have been discussed [Tobias (267)]. Axoplasmic mitochondria are numerous and of variable size, geometry, and distribution; circumferential arrangement, often strikingly regular, is common in squid [Geren & Schmitt (114)] and lobster [Tobias (269)]. (See also receptoneural junction.) Vesicles, comparable to those seen at junctions, are perhaps more numerous in subnodal axoplasm [Robertson (217); Nelson (193)].

Mechanical, optical, and thermal properties.—This area has been reviewed by Tobias & Nelson (24). Correlation of the temperature coefficient for linear expansion with electrical activity is doubtful. A high Q_{10} and high energy of activation for the rate-controlling step in activity of *Electrophorus electricus* electroplax is said to mean that "chemical processes (are) responsible for the specific changes in permeability of conducting membranes during activity" [Schoffeniels (224)]. Experiments of Kayushin *et al.* (149), together with x-ray data, suggest that failure of excitability and a shift in mechanical properties at 30 to 35°C. reflect a change in a nonmyelin component. Thermal block of nerve is counteracted by anodal polarization, by calcium, or by hypertonicity [Cerf (60)]. Each of these deblockers appears to the reviewer as a structure-condensing agent. Since temperature rise, in the right range, can decrease protoplasmic viscosity in certain forms, i.e., disperse structure, the deblockers may counteract the effect by favoring reassociation. Here, too, birefringence studies could be profitable (see previous discussion of stretch). That such deblockers may raise the resting potential, while thermal block lowers it, is no satisfactory "explanation". The notion that all is explained when one finds a voltage component in a phenomenon is an erroneous conception subscribed to by many. A polarizing electrode, calcium, or hypertonicity do much more than just change potentials [Tobias (267)].

Sheath tension may play a part in proximodistal axoplasm flow [Lubinska (174)], which increases in whole nerve with activity [Vodička (278)]; it is not clear whether the movement is intra- or extra-axonal. End-plate volume is also said to increase. Contrary experiments are summarized by McIntyre (183). Vacuoles can move along the axoplasm toward cell bodies, at least in cultured cells [Nakai (191)]. Little-known interferometric experiments show that frog nerve diameter changes with activity [Kayushin & Lyudkovskaya (147, 148)], as does rigidity [Kornakova *et al.* (156, 157)]. *Carcinus* nerve shortens reversibly with activity; one wonders if nerve is muscle, mechanically much attenuated. The light-scattering change in active nerve is much prolonged by azide, but its onset is unchanged; it is therefore caused by events associated with activity, not with recovery. Analysis of correlated light-scattering and length changes with activity makes it appear highly unlikely that the light-scattering changes reflect only shifts of water between intra- and extracellular phases [Bryant & Tobias (50)].

New instruments show that impulses in *Maia* nerve are accompanied by initial heat production, possibly associated with the active impulse phase, and followed by slower heat absorption. Part of the heat produced may result from Na-K exchange, i.e., be a heat of mixing. One still does not know how heat changes relate in time to the rising and falling phases of the action potential [Abbott *et al.* (28)]. This might be resolved if some technical advance would allow the use of *Nitella* where the action potential lasts so long, but the surface to volume relation is unfavorable for sufficient heat production to be detected even by the best methods. The need for using many fibers, with impulses reaching the recording device at different times, also poses major difficulties for temporal analysis.

In addition to stretch and temperature as tools in the analysis of ultrastructural parameters of function, hydrostatic pressure is also much neglected [for apparatus see Downing & Wilkie (86)]. Increasing the pressure on frog axons mainly prolongs the falling phase of the spike, increases rheobase but little, and has practically no effect on conduction velocity or spike amplitude. Squid axons show a threshold fall and spontaneous firing at high pressure [Spyropoulos (240, 241)]. This latter might be expected in terms of equating high pressure with structural colloid dispersion (solation) and this, in turn, with excitation. Failure of capacity to change even when resistance falls to irreversible levels only implies that a small change in structure can produce a large resistance change. Ultrasound as an analytical tool is promising and begins to be explored (46, 109, 280, 289).

Ion selection; metabolism.—How cells segregate ions and what, in terms of ion selectivity, differentiates the active from the resting state are paramount issues. Extensive treatments have appeared (1, 10, 13, 17, 18, 22).

In re a metabolic parameter: adenosinetriphosphate (ATP) dephosphorylation seems important for maintaining low [Na] in squid nerve, arginine phosphate being used for resynthesis; injection of ATP or arginine phosphate into cyanide-poisoned squid axons partly restores sodium efflux [Caldwell and Keynes (58, 59)]. Anaerobiosis, dinitrophenol (DNP), azide,

or chloretone interfere with frog nerve ion distribution [Coelho (65); Hurlbut (134)]. A favored substrate for *Carcinus* nerve adenosinetriphosphatase may be sodium-magnesium-ATP. In the presence of Na, K, Ca, and Mg in roughly axoplasmic concentrations, increase in Na or Ca or decrease in K intensifies enzyme activity [Skou (235)]. The evidence is of the "compatible with" sort, but were the substrate somehow to bind some sodium, then such reactions could make sense for known ion exchanges. In this context one recalls that excitation may inhibit frog nerve phosphorylations [Abood & Goldman (30)]. Pappius & Elliott (198) show dependence of brain slice potassium on oxidative metabolism. The resting potential in excised guinea pig and cat cortex, kidney, and liver depends on oxygen [Li & McIlwain (163)]. Brink (19) discusses glycolysis and respiration in maintaining frog nerve ion concentrations, but "it has not been possible to demonstrate that the extra oxygen metabolism of frog A-fibres is required as an energy source for restoring the ionic changes of activity." Several experiments suggest a role of mitochondria in ion regulation [Berger (43); Gamble (113)]. That sodium transport requires active cholinesterase on the side of the cell surface from which the ion is transported is attested by experiments of Van der Kloot (275a).

Although the above and older experiments demonstrate association between metabolism and ion segregation, none comes even close to disclosing mechanically linked, molecular level events coupling metabolism to ion transport or accumulation. In this context see also Ling (165).

One comes to a consideration of ion selection by binding. Thus, the presence of hyaluronic acid in axoplasm has not been confirmed though such material might act as an ion exchanger [Abood & Abul-Haj (29)]. Folch *et al.* (19) consider acidic lipides possible cation binders. Human blood yields a material, probably phosphatidic, which binds more potassium than sodium; phosphatidylserine, sphingomyelin, and acetal phosphatide also discriminate; cholesterol does not [Solomon *et al.* (238)]. Neither collagen nor cephalin content of transected nerve is paralleled by cation content, implying no binding to these substances [Krnjević (158)]. Neither adenosine-, inosine-, guanosine-, uridine-, or cytidine triphosphate, nor TPN or isethionic acid, as molecules free in solution, selectively binds Na or K [Tosteson (271a)], but sodium in calf thymus nuclei is said to be 30 per cent associated with nucleic acid, protein, and lipide [Itoh & Schwartz (141)]. Thus nucleotide might participate in transport mechanism when bound to a macromolecule, though not when free. Glycerol-extracted muscle contains and accumulates slightly more sodium than potassium, according to Fenn (97). Similar results are reported for distilled-water-soaked or sucrose-treated muscle [Tobias (266); Steinbach (244)]. Glycerol-extracted muscle supplied with relaxing factor and calcium accumulates, on the other hand, slightly more potassium than sodium [Baird *et al.* (36)]. Ling (166) has developed a picture of how protein groups can change ion preference; no full description is available. Potassium is displaced from some cells by amino acid (62, 90, 202, 207).

Calcium has been known to be largely bound in nerve, in part probably

to a residually negative, anodally migrating particle [Solomon & Tobias (239)]. In brain it may be bound to diphosphoinositide [Folch (105)]. ^{45}Ca injected into squid axons had less than 1/45 of free solution mobility [Hodgkin & Keynes (132)]. No anodal migration was detected; perhaps anodally migrating Ca is extraaxonal; another possibility is that Ca injected as CaCl_2 may not combine with the same anions or anionic groups as does Ca normally incorporated.

Metabolic experiments not directly related to ion balance follow. Squid axon sheath, mainly Schwann cells and connective tissue, respire and uses many substrates at a high rate, indicating that many data on metabolism previously ascribed to the axon *per se* should be attributed to sheath elements [Schmitt & Geschwind (20)]. Nodal block by neopyrithiamin but not by oxythiamin suggests thiamin is important for excitation otherwise than in decarboxylations, but one cannot yet pinpoint the metabolic lesion [Kunz (161)]. Reversal of ultraviolet injury to nerve by visible light reveals no action spectrum pointing clearly to any one damaged absorber [Pierce & Giese (201)] suggested earlier to be thiamin. No convincing change in ultraviolet absorption by frog or lobster axons is seen with activity [Eggen (95)]. Giese (116) describes photodynamic effects of *Blepharisma* pigments on frog and crab nerves. Protein, in peripheral nerve, reversibly denatured during activity (192, 273, 274), could contribute to the light-scattering changes with impulses [Bryant & Tobias (50)].

*Electroionics.*⁴—Resting potential genesis is not yet clear. Conway (1) and Shanes (22) review applicability of the Nernst and Goldman equations. As partial tests, the injury potential of rabbit nerve is an approximately linear function of temperature [van Harreveld & Christensen (276)], and the resting potential of squid axon *in situ* is somewhat higher (av. 77 mv.) than after excision [Thies (265)].

Fundamental is the problem of whether K_i is free or bound, i.e., whether the activity coefficient of K_i is the same as of K_o . Recent efforts to find binders for K are listed in the preceding section. Conway (1, 18) and Ling (18) detail and discuss the problem. Only three issues will be noted here. (a) Since closeness of approach of K and Na to fixed, anionic sites may be crucial, estimates of hydrated ion size become critical; which values are correct is not yet clear [Bell (41)]. (b) The extent to which binding, as proposed by Ling to account for K accumulation, would reduce K_i activity and thus change E_K and osmotic pressure is uncertain. Finally, (c) even if preferential protein binding segregates K from Na, there remains the question of how NaCl is excluded from the larger part of the intracellular water space. One needs more data on molecular geometry of cell solids *in vivo* and on ion diameters, as well as better insight into the state of K_i than is gained from mobility [Hodgkin & Keynes (130)] or water shift experiments [Relman (209)]. One needs to consider usually neglected parameters, as in suggestions of Szent-Györgyi (258) concerning cell water. If intracellular ions and water

⁴ A term coined by K. S. Cole in 1954.

are compartmented [Cowie *et al.* (68)] additional problems arise. Shaw *et al.* (229) and Simon *et al.* (233) reject maintenance of K_i by a Donnan equilibrium in favor of partial "binding" to an ordered intracellular phase; a second phase, *ca.* 1/30 the cell volume, contains Na, K, and Cl in equilibrium with external solution.

Attempts to clarify the issue by changing tissue ion composition and examining its correlation with electrical potentials meet difficulties even when isolated cells are used; the more often used multicellular masses make still more problems. Thus, muscle is soaked in some solution, a microelectrode measures potentials, the muscle is analyzed, K_i is derived and the fit of the data to the equations is evaluated. A problem then arises because potential measurements are probably made on superficial cells, whereas whole muscle is analyzed, and, even in the sartorius, unless soaking is prolonged, superficial cells may attain one composition, deeper cells another. Potential measurements on one population may be compared with composition of another [Conway (1); Harris & Steinbach (126)].

Thus, Shaw *et al.* (229) found no correlation of potential with ionic gradients in toad muscle. The muscles were soaked 1 to 3 hr., but unpublished experiments by the reviewer on equilibration with distilled water suggest this may not be long enough or just borderline. Stephenson (255) soaked frog sartorii 11 to 18 hr. in K-free solution, long enough, and found that during 50 min. recovery in 10 mM KCl, while K was being regained, the potential remained nearly constant at 40 mv. While large net fluxes occurred, the Nernst equation was not satisfied. Quite aside from whether it should even theoretically be applicable under such conditions, the failure of correlation between potential and reaccumulated K may not be crucial, because the emf. was compared with $[K]_i$ after only 50 min. soaking. The negative slope of the relation of K_i to emf. after such soaking might mean that superficial fibers used for emf. measurement gained relatively more K the greater the initial depletion, but, because of the short soak, K measurement on the whole muscle would yield lower values the greater the initial depletion. The findings deserve clarifying and may be vindicated; as they stand they are of less value than they potentially may be. That the emf. had a tendency to change toward 40 mv. during recovery, and the suggestion that the emf. in distilled water-soaked muscle [Tobias (266)], the B -emf. of Ling & Gerard (167), and the emf. deemed by Stephenson to be independent of K, Na, and Cl have a common origin are most interesting matters.

Experiments on effects of microinjected ions on the emf. in squid axons do not suffer from the same problems [Hodgkin & Keynes (131)]. However, here the question of extent and uniformity of distribution of injected material comes up, as does the point that a cation injected with some anion may not end up in the same state as when it enters by its normal transsurface path.

The unclear status of how transsurface potentials arise is added to by the following. That practically the same potentials are recorded with Sb as with Ag-AgCl in KCl electrodes is interpreted to mean the resting potential is not a Donnan one, but results from "active processes in the membrane"

[Kao & Grundfest (143)]. Electrical potential data do not fit the equations exactly for eel electroplax or lobster axons [Altamirano & Coates (31); Dalton (72)]. Experiments of Schoffeniels (225) show K_o to have different effects on the innervated and noninnervated surfaces of the single isolated electroplax. *Fundulus* egg [Kao (142)] and *Nitella* [Kishimoto (150)] studies are interesting but not yet greatly clarifying. Stämpfli & Willi (243) find a residual 10 mv. or less emf. after electrical destruction of the nodal "membrane". That toad muscle action potentials do not change with the Na gradient as predicted by theory is reported by Shaw *et al.* (229), but Hodgkin (13) suggests errors arise because of difficulty in measuring $[Na]_i$, and because experiments with raised $[Na]_o$ should have been controlled with tissues in a similarly hypertonic solution, since high $[Na]_o$ will raise $[Na]_i$ by removing water.

Replacement of Na_o with preservation of excitability is extended to frog A-fibers by the use of guanidinium and other quaternary ammonium ions (74, 80, 162). Müller (189) [quoted by Hodgkin (13)] reports that "after the passage of a large outward current a node of Ranvier will apparently give a prolonged action potential in the complete absence of Na or K from the external medium." Such experiments are greatly interesting, but none invalidates *per se* the notion that sodium when present in certain forms, carries the inward part of the action current. Thus, recent experiments with myelinated nerve are compatible with the hypothesis [Frankenhaeuser & Persson (108)]. However, sodium may not be a universal carrier of inward current. In the phytophagous insect, *Carausius*, muscle action potentials, though modifiable by Na_o , persist even when Na_o is zero. Sodium appears to affect excitability without being a specific current carrier [Wood (285)]. There may be a complication in the case of insects because of semipermeable pericellular sheaths. What these experiments and older ones like them do mean is that certain chemicals can to a degree substitute for sodium in some forms, and in others sodium may normally not be the carrier of inward current. Therefore the ubiquity, specificity, and selectivity of any single mechanism permitting current entry are probably less than many have assumed.

Tetraethylammonium ions injected into squid axons prolong the action potential which then resembles that of Purkinje fibers. A peak is followed by a plateau during which membrane resistance is but little displaced from the resting level [Tasaki & Hagiwara (262)]. It is argued there are two stable membrane states, and initiation or abolition of action potentials represent transitions. A cobalt electrode model has properties consistent with this view according to Tasaki (259). Similar prolongation of the action potential of neuron somata has been reported [Eccles (4); Koketsu *et al.* (153)]. Production of high frequency, oscillating, transsurface currents in voltage clamped squid axons and toad nodes, by subthreshold depolarization, is said to imply that the "mechanism of production of action potential is very different from what has hitherto been generally accepted" [Tasaki & Bak (260, 261)]. One wonders if it may not simply represent bringing transiently and approximately into phase, random micro-area-oscillations in

transsurface current and potential, which have not yet been individually detected and are related perhaps to quantal subthreshold responses at the node of Ranvier [Del Castillo & Suckling (77)].

Initial after-impedance and the major early impedance change are differently sensitive to temperature and to K_o [Amatniek *et al.* (32)]. Perhaps they therefore reflect different underlying changes in the cell surface. Attempts to explain the action of K_o in terms of potential are unsatisfactory. The questioning should probably be pushed back one step: "What kind of effect might potassium have on the structure of the surface components which then could secondarily produce transsurface potential and conductance changes?" Ions are so often thought of only in terms of direct action on potentials. That they also produce chemical and structural changes which can secondarily affect potentials is rarely considered.

Apropos of calcium there is an intermediate $[K]$ at which sensitivity to calcium-lack is maximal [Posternak *et al.* in (19); (222, 242)]. This non-linearity suggests that potassium has two effects, one of which dominates at first to be overtaken by the second; hence explanations purely in terms of potential will probably be unsatisfactory here too. Ichioka & Konishi (136) report effects of calcium on nodes and Frankenhaeuser & Hodgkin (107) on squid axons. Fivefold Ca_o increase is equivalent to 10 to 15 mv. hyperpolarization, and in general

changes in Ca concentration and changes in membrane potential have similar effects on the systems which allow Na and K to move through the membrane during the spike. However, Ca has an excessively large effect on anodal shutting off of sodium conductance.

One should recall that an anode has effects beyond that of electrical polarization. Axonal shrinkage anodally could add to or subtract from alterations produced by ions alone [Tobias (267)]. Greatly interesting is the fact that external Ca ions seem immediately necessary for conduction; the mechanism is not clear [Frankenhaeuser (106)].

At the frog node, depolarization by excess K was accompanied by threshold rise, but depolarization electrically was accompanied by threshold lowering. The argument that low membrane resistance caused by excess K could raise rheobase was rejected because K lack, which should presumably have an opposite effect, also raised rheobase [Hashimura & Wright (127)]. One wonders if excess K acts perinodally to broaden the current path, thus decreasing current density with an apparent rise in threshold. At any rate, because of the findings and results with Ca, it is suggested that binding of Ca to certain sites, competition for these sites by all cations, and possible effects of depolarization on occupancy of these sites make Ca a key ion in excitability [see also Tobias (267, 269)].

Resting flux of ^{45}Ca in squid axons is small compared with other ions; activity increases uptake [Hodgkin & Keynes (132)]. One wonders if Ca enters axoplasm with impulses or exchanges with surface-bound Ca. Radioautographs might resolve this.

The excitation-contraction coupling hypothesis of Csapo & Suzuki (71) is noted here, since long-axis Ca and K separation may be crucial in nerve excitation [Tobias (267)]. It is suggested that the action potential penetrates along Z bands, changing charge nearby and releasing Ca ions; internal currents move this Ca longitudinally to activate the contractile system. A difficulty is the background of evidence against a primary role of longitudinal current in contraction. In this general context, one also notes that ultraviolet radiation produces contracture [Punt *et al.* (203)], possibly by releasing Ca, and decreases retention of potassium by yeast [Bruce (49)], perhaps by a similar process.

Insecticide studies go extensively into insect nerve electrophysiology [Yamasaki *et al.* (286, 287)]. A series of papers deals with electrophysiology of crayfish inhibitor and excitor fibers [Zacharova (288)]. Electrophysiological changes following activity are described by Ritchie & Straub (213) and Gasser (113a), anodal break excitation of nodes by Ichioka (135), effect of applied current on nodal action current by Konishi (154), and relation between fiber dimensions and conduction velocity by Thiel (264) and Cragg & Thomas (69). A new isolated electroplax preparation is described; tertiary and quaternary compounds are said to compete for the same receptor [Schoffeniels & Nachmansohn (226)]. A hyperpolarization spike follows earlier small depolarization in a protozoan [Hisada (129)]. However, outward current excites, inward current does not; the latter hyperpolarizes and inhibits spikes. Thus excitability resembles that in nerve, but the spike is reversed. Kirk & Falk (151) describe a simple technique for making micro-electrodes.

Membrane concept.—A unique membrane whose state is synonymous with rest or excitation is taken for granted by many. Its estimated thickness is such that electron microscopy should reveal it. Perhaps it has, but one cannot point to any one electron dense layer and say "This is the excitable membrane." Certainly the ubiquity of a common type of surface structure [Robertson (219)] does not establish identity of a functionally metastable membrane, even when only one electron dense layer is seen. The essence of the membrane concept, *in re* excitability, and the main criterion for recognition, is controlled instability, i.e., useful interconvertibility between resting and active states of a structural complex continually exposed to movement, distortion, and strain; electron microscopy has not yet seen this. Fixed tissue conveys static structure, but cell surface units are certainly not rigidly fixed in life. Microlocal chemical changes and random thermal agitation must change such structures here and there continuously so that "the" membrane is more likely to be a number of membranes, or regions, or different kinds of changing sites, or appearing and disappearing portals of entry and egress depending on chemical and physical events nearby. This is not the same as implied by a mosaic which can also be static. Here one refers to continual change, and if one includes the mosaic then it too will continually change.

It is argued [Tobias (269); Tobias & Nelson (24)] that the innermost,

osmophilic axon surface layer (axolemma) is not yet demonstrated to be different from similar layers farther out, or from myelin, whether in invertebrate or vertebrate axons. Uniqueness can, however, be conferred by position; the innermost layer is the only one bounded by axoplasm on one side and nonaxoplasm on the other, and some layer farther out is the only one bounded by tissue fluid and nontissue fluid; and one knows that interfacial film properties depend as much on what contacts them as on composition and structure. These authors also propose a surface structure model which can, to a degree, be tested, and speculate on its interactions with potassium and calcium in excitation triggering. It is suggested that protein may penetrate the still excitable axon; essentiality of phospholipide integrity and dispensability of some surface protein are demonstrated.

Segal (228) proposes an excitation theory involving changes in protein coacervates. Mullins (190) and Burgen (55) suggest variants of the pore size hypothesis of permeability.

Hemolysis by, and effects on muscle-resting potential of, surface active agents are pertinent [Rideal & Taylor (210); Wasano *et al.* (279)], as is work of Mercer (185) and Ries & Kimball (211, 212) on electron microscopy of protein and fatty acid monolayers. Effects of cardiac glycosides on red cells suggest experiments on excitability because of the role of steroid structure [Glynn (117)].

SYNAPSES AND CELL BODIES

Structure and composition.—Summaries or bibliographies, or both, of electron microscopic findings are given by Fernández-Morán (19), Eccles & Jaeger (89), and De Robertis & Vaz Ferreira (82). The synapse is, in general, two osmophilic layers, each some 50 Å thick, separated by a 100 to 200 Å cleft; presynaptic axoplasm contains mitochondria and usually large numbers of vesicles 300 to 400 Å in diameter. Recall postjunctional vesicles at some receptoneural junctions discussed at the beginning of this chapter. In some cases the vesicles tend to align in chains interconnected by irregular tubules [Palay in (26)]. Earthworm synapses have different numbers of membranes, perhaps depending on size [Issidorides (138)]. Schultz *et al.* (227) find the roles of both synaptic membranes and vesicles doubtful, since they sometimes see "synapses" with astrocytic processes. The obvious suggestion that the vesicles may be carriers of a chemical transmitter has not yet been investigated.

In cerebral cortex and callosum, glia and cell processes are said to take up all available room with a fairly constant interspace of about 200 Å [Schultz *et al.* (227)]. If this is true, then it is not easy to see how light microscopy can resolve muco- or glycoprotein ground substance "occupying the spaces between the elements of the cortical tissue" [Goodhead (119)]. However, such findings must somehow be considered in any evaluation of electric field effects [Terzuolo & Bullock (263); Grundfest (121); Köhler & O'Connell (152)], cell movements [Geiger in (26)], growth, and regeneration. Whiting

(283), Malhotra (179), and Chou (61) describe Mauthner cells and neuronal inclusions. Studies of cultured neurons [Nakai (191); Geiger, in (26); Hild (128)] should gain meaning from monitoring their electrical activity [Crain (70)]; such cells could also be studied by a powerful combination of electrophysiological, optical, and electron microscopic techniques and by microchemical ones [Lowry in (26)].

Splanchnic nerve stimulation produces either a rise or a fall in vesicle number in adrenal nerve endings, activity apparently increasing both formation and disappearance, one or the other dominating depending on amount of activity [De Robertis & Vaz Ferreira (82)]. In some nerve endings of severely dehydrated animals, the vesicles and tubules largely disappear to be replaced by a fibril feltwork [Palay in (26)]. Activity produces only a transient increase in soma size [Edström (92)]. This is of interest, though not crucial, for the idea that increases in synaptic contact area, by swelling, might influence learning [Tobias (268); Eccles (88)]. Failure to find lasting dimensional changes weakens such a view but small changes might elude conventional cytology. For a review of the electrophysiological approaches to the learning problem see Burns (56). Eccles & Jaeger (89) consider synapse geometry and dimensions. For certain types of synapses "diffusion satisfactorily accounts for the observed rates of removal of the transmitter from the synaptic cleft"; invaginations in the photoreceptor-neuron synapse serve to slow removal by diffusion since, it is argued (89) on highly tenuous evidence, "the liberation of the presynaptic vesicles is effected by . . . hyperpolarization, which is much smaller than the depolarization that appears to be the causal factor for the liberation from presynaptic endings on muscle"; synaptic clefts are said to offer negligible resistance to postsynaptic currents.

Cholinesterase is present in some sympathetic and spinal ganglion cells and in all anterior horn cells, decreasingly in the order soma, axon, dendrites, and nucleus, with nucleolus containing negligible amounts [Giacobini (115)]. Canine neurons contain mucoproteins, less in young animals [Sulkin (256)]. Nissl substance, rich in nucleoprotein, pre-exists in cultured chick neurons [Deitch & Moses (75)]. The ultraviolet action spectrum for inhibition of "regeneration" of embryo ganglion cells in culture does not coincide with the absorption spectrum, maximum absorption being at 265 $m\mu$., maximum effect at 248 $m\mu$. [Bammer (37)]. Single nerve cell analyses show great excess of certain enzymes. This is considered a device for intracellular homeostasis [Lowry in (26)]. Such microtechniques allow assessment of cell variation, will eventually extend to cell parts [Giacobini (115)], and may remove uncertainties stemming from measurements on tissue masses containing mixed cell types and extracellular elements. Anderson & van Breemen (33) find that malanonitrile produces changes in Nissl bodies, Golgi complex, and mitochondria, but earlier observations on an increase in Nissl substance are not confirmed.

Transmission and conduction.—Only few issues thrust forward in recent

years can be considered. A chemical mediator of inhibition was sought in 1936 by Pantin (197) who depended on diffusion to carry the inhibitor from one *Carcinus* leg to another bound to it.

Out of some twenty successful experiments four gave evidence of inhibition of the second muscle following excitation of the inhibitor nerve of the first. . . . Since the large proportion of experiments did not show any obvious effect, the experiments are not conclusive.

Perhaps these experiments demonstrated an inhibitory mediator; and some technical change, as perfusing two legs in tandem, might have increased positive results. Babsky (35) showed increase in cortical cholinesterase activity under an anode. One wonders if a comparable phenomenon might occur at cholinergic synapses to produce inhibition. Since then, extracts of neural tissue have been shown to mimic inhibition in some ways (96, 103, 104); apparently the major active substance is γ -aminobutyric acid (38, 48, 184). Lissak & Endrocz (168) also find inhibitory substances in brain. How γ -aminobutyric acid (GABA) acts is certainly not known in any molecular terms; it may increase conductance in the synaptic region of the crayfish stretch receptor thus shunting electrotonic current to the higher threshold part of the cell [Edwards & Kuffler (93)]. In the cortex there is evidence that it blocks depolarizing postsynaptic potentials [Purpura *et al.* (204, 205)]. Certain crucial data are lacking: there is no evidence that the material is released when inhibition occurs, and no specific substance has been found which would limit its action, as does cholinesterase for ACh; conceivably, local pH changes could control GABA level by making either glutamic decarboxylase or GABA transaminase dominantly active [Roberts *et al.* (214)]. At the moment it is an interesting substance which cannot yet be accepted or rejected as a chemical mediator. Kuno (160) has obtained further evidence that strychnine acts by depressing inhibition. Sheveleva (232) concludes that there is no reason to ascribe inhibition or excitation to any one group of fibers in the nervous system and that response type depends on the functional state of the receiving substrate.

The electrical properties of toad spinal ganglion cells are enumerated by Ito (140). Actions of histamine, pilocarpine, and 5-hydroxytryptamine on ganglion cells are described by Trendelenburg (272). Fuortes *et al.* (110) confirm that somatic excitation is initiated at the axon hillock and that the rising phase inflection of the spike is decreased by depolarization and increased by hyperpolarization. Coombs *et al.* (66) further describe motoneuron impulses. A point of maximum interest here is the as-yet-undisclosed basis for soma-dendritic threshold being several times that of the initial segment. Eccles (4) suggests that "either glial coverage or some intrinsic difference in the membrane . . . must . . . be invoked." One wonders if this could be settled by microelectrode studies on cultural neurons, some of which are naked (Geiger, R., personal communication). An apparent exception is the neuron of the roach in which " . . . the rheobase of the axon is much

higher than that of the soma" [Yamasaki & Ishii (287)]. Hagiwara & Bullock (123) report electrophysiological studies of lobster cardiac ganglion cells.

In 1955 Bullock & Hagiwara (52, 53) concluded that for the giant synapse of the squid "... current cannot be the synaptic transmitter"; in 1956 Grundfest *et al.* reported a similar conclusion *in re* cortical dendrites (122). Since then Grundfest has written exclusively on the problem both with respect to electric organs (9) and central nervous system synapses (8). Kao & Grundfest (144) detect both spike activity and postsynaptic potentials which can excite the giant septate axon of the earthworm to repetitive activity. The postsynaptic potentials apparently originate at a distance in the neuropile, not at the septa.

Such matters as analysis of spreading depression [van Harreveld & Ochs (277); Burešová (54)], origin and spread of electroencephalographic rhythms [Belkering *et al.* (40); Bremer & Stoupel (47)], and excitation of certain brain regions by hypertonic media [Clemente *et al.* (64)] are interesting and appropriate for this review, but space precludes their consideration.

NEUROEFFECTOR JUNCTION

Structure.—Electron microscopy of neuroeffector junctions is largely electron microscopy of neuromyal junctions. In *Anolis*, multiple, complexly folded troughs in the muscle fiber surface are occupied by axonal termini; a quintuply layered structure ("membrane") 500 to 700 Å thick separates axoplasm from sarcoplasm. Terminal axoplasm is said to contain "vesicular or tubular appearing structures" 300 to 500 Å in diameter [Robertson (215)]. Reger (208) has studied the normal and denervated mouse neuromyal junction. Normally the synaptolemma is 500 to 600 Å thick with five differentially osmicated layers. The postsynaptic sarcolemma is deeply infolded forming a series of lamellae. Vesicles are seen on both sides of the junction, but there are fewer postsynaptically. The neuromyal junction in the wasp lacks the complex infolding seen in vertebrates and contains, in addition to presynaptic vesicles and mitochondria, many postsynaptic granules [Edwards *et al.* (94)]. The smooth muscle junction in the mouse is described by Caesar *et al.* (57).

Electrophysiology.—The comparative physiology has been reviewed by Hoyle (14). Work of Wood on an herbivorous insect, in which the blood ratios of Na:K and Ca:Mg are less than 1, has already been noted in the section on Electroionics. In addition it was found that although Ca is necessary for development of the junctional potential, Mg block is not antagonized by Ca. Furukawa *et al.* (112) find that ACh depolarizes toad muscle endplates in the absence of Na. Liley (164) has investigated the multiquantal junctional responses probably caused by synchronized discharge of several quanta of transmitter, each of which may be equal to the quantum normally generating a so-called miniature end plate potential. These potentials may be responsible for fibrillation in muscles treated with anticholinesterases. In this context, because of differences in tubocurarine inhibition of tetanic contractions pro-

duced by different cholinesterase inhibitors, even within species, Axelsson *et al.* (34) conclude that something other than cholinesterase inhibition with ACh accumulation must occur. Dybing (87) finds an unexplained action of epinephrine in temporarily relieving neuromuscular block produced by Mg, and Furukawa (111) concludes that procaine has a curarelike action in desensitizing the end plate to ACh, a Mg-like action in decreasing output of ACh from endings, and an anaesthetic action decreasing muscle membrane excitability.

In a series of four papers, Del Castillo & Katz (76) describe a method for iontophoretic application of chemicals to the end plate, confirm the classical view that curare is a competitive inhibitor combining reversibly with end plate receptors, conclude that neuromyal junctional receptors are readily accessible to applied drugs, suggest that the time course of certain drug effects depends on rate of access and hydrolysis rather than kinetics of drug-receptor reaction, and describe interaction of certain choline derivatives with the end plate. More interestingly perhaps, Katz & Thesleff (145) have investigated the phenomenon of desensitization of the end plate by ACh. Even small doses of ACh which depolarize 0.5 to 1 mv. if maintained for 10 to 20 sec. can produce up to 50 per cent desensitization, and conditioning doses giving depolarization of 10 to 20 mv. can give 100 per cent desensitization. On withdrawal, sensitivity starts to recover with a half time of *ca.* 5 sec. The mechanism of desensitization remains unknown.

A COMMENT

The molecular-level structural parameter in conduction and transmission is clearly of great importance and interest. Modern, high resolution techniques are only beginning to be applied. Electron microscopy, x-ray diffraction and fluorescence, absorption spectroscopy in the ultraviolet and infrared, light scattering, polarized light, nuclear magnetic resonance (23), etc. are still esoteric enough and far enough removed from the everyday tools used by most biologists so that such techniques are still employed mainly by specialists and largely for what they can reveal by themselves. Only rarely is their use combined with the more prosaic monitoring of potentials, excitability, conduction and transmission, so-called membrane constants, etc., etc. As a result the description of ultrastructure is, as is probably inevitable in such an infant field, far ahead of its correlation with function. This correlation is coming, however, and one feels that there will be a gradual shift from emphasis solely on structure, on chemical events, or on electroionics toward attempts at integrating all three.

A major stimulus comes from a consideration of research in muscle physiology. Here, although the electrical properties and changes with activity are qualitatively essentially like those in nerve, the more obvious phenomena are structural in nature. Therefore, the chemical approach has always been oriented toward understanding structural changes, and progress has been impressive. In nerve, because structural changes with activity are small and were long overlooked, and because electronic devices are so power-

ful, the emphasis has always been on trying to understand the chemistry directly in terms of electrical events. In the case of both muscle and nerve it would be healthy if more attention were paid to trying to understand the electrical events also in terms of structure and structural changes. Recently, because of the possibility that action currents represent passive redistribution of ions, efforts have been oriented more toward trying to understand nerve metabolism in terms of ion selections and segregations produced by so-called active transport. This research is probably all to the good, but these efforts still may be a bit misguided. A more productive question might be "How is the cell chemistry used to maintain and repair, at a molecular-macromolecular level, metastable structures, which are then in turn responsible for the ion distributions of the resting state and which can change in a reversible fashion so as to permit the transient ion movements which are the electrical expression of the active state?" Obviously this will be a difficult question to answer. The structural changes which take place with activity in nerve are infinitesimally small as compared with those in muscle. However, in the writer's opinion the important point is the need to recognize that both the electrical events and metabolic activities will one day make most sense when referred to molecular-macromolecular structure and to the structural-organizational changes occurring when the cell goes from a state of rest to that of its characteristic activity and back again.

LITERATURE CITED

Summaries, reviews, monographs and symposia—

1. Conway, E. J., *Physiol. Revs.*, **37**, 84 (1957)
2. Cranefield, P. F., and Hoffman, B. F., *Physiol. Revs.*, **38**, 41 (1958)
3. Davis, H., *Physiol. Revs.*, **37**, 1 (1957)
4. Eccles, J. C., *Physiology of Nerve Cells* (Johns Hopkins Press, Baltimore, Md., 270 pp., 1957)
5. Engström, A., and Finean, J. B., *Biological Ultrastructure* (Academic Press, Inc., New York, N. Y., 326 pp., 1958)
6. Geiger, A., *Physiol. Revs.*, **38**, 1 (1958)
7. Grundfest, H., *Ann. N. Y. Acad. Sci.*, **66**, 537 (1957)
8. Grundfest, H., *Physiol. Revs.*, **37**, 337 (1957)
9. Grundfest, H., *Progr. in Biophys. and Biophys. Chem.*, **7**, 1 (1957)
10. Harris, E. J., *Transport and Accumulation in Biological Systems* (Butterworths Scientific Publ., Ltd., London, Engl., 291 pp., 1956)
11. Hebb, C. O., *Physiol. Revs.*, **37**, 196 (1957)
12. Hecht, H. H., *Ann. N. Y. Acad. Sci.*, **65**, 655 (1957)
13. Hodgkin, A. L., *Proc. Roy. Soc. (London)*, [B]**148**, 1 (1958)
14. Hoyle, G., *Comparative Physiology of the Nervous Control of Muscular Contraction* (Cambridge University Press, Cambridge, Engl., 147 pp., 1957)
15. Hughes, J. R., *Physiol. Revs.*, **38**, 91 (1958)
16. LeBaron, F. N., and Folch, J., *Physiol. Revs.*, **37**, 539 (1957)
17. Lundberg, A., *Physiol. Revs.*, **38**, 21 (1958)
18. Murphy, Q. R., Editor., *Metabolic Aspects of Transport Across Cell Membranes* (University of Wisconsin Press, Madison, Wis., 379 pp., 1957)

19. Richter, D., Editor, *The Metabolism of the Nervous System* (Pergamon Press, London, Engl., 599 pp., 1957)
20. Schmitt, F. O., and Geschwind, N., *Progr. in Biophys. and Biophys. Chem.*, **8**, 166 (1957)
21. Schwan, H. P., *Advances in Biol. and Med. Phys.*, **5**, 148 (1957)
22. Shanes, A. M., *Pharmacol. Revs.*, **10**, 59 (1958)
23. Sogo, P. B., and Tolbert, P. M., *Advances in Biol. and Med. Phys.*, **5**, 1 (1957)
24. Tobias, J. M., and Nelson, P. G., in *A Symposium on Molecular Biology* (Zirkle, R. E., Ed., University of Chicago Press, Chicago, Ill., 360 pp., 1958)
25. Ungar, G., *J. physiol. (Paris)*, **49**, 1235 (1957)
26. Waelsch, H., Editor, *Ultrastructure and Cellular Chemistry of Neural Tissue*, II (Paul B. Hoeber, Inc., New York, N. Y., 249 pp., 1957)
27. Whitfield, I. C., *Progr. in Biophys. and Biophys. Chem.*, **8**, 1 (1957)

Research reports.—

28. Abbott, B. C., Hill, A. V., and Howarth, J. V., *Proc. Roy. Soc. (London)*, [B]**148**, 149 (1958)
29. Abood, L. G., and Abul-Haj, S. K., *J. Neurochem.*, **1**, 119 (1956)
30. Abood, L. G., and Goldman, E., *Am. J. Physiol.*, **184**, 329 (1956)
31. Altamirano, M., and Coates, C. W., *J. Cellular Comp. Physiol.*, **49**, 69 (1957)
32. Amatniek, E., Freygang, W., Grundfest, H., Kiebel, G., and Shanes, A. M., *J. Gen. Physiol.*, **41**, 333 (1957)
33. Anderson, E., and van Breemen, V. L., *J. Biophys. Biochem. Cytol.*, **4**, 83 (1958)
34. Axelsson, J., Gjone, E., and Naess, K., *Acta Pharmacol. Toxicol.*, **13**, 319 (1957)
35. Babsky, E. B., *Nature*, **157**, 730 (1946); **158**, 343 (1946)
36. Baird, S. L., Karrman, G., Mueller, H., and Szent-Györgyi, A., *Proc. Natl. Acad. Sci. U. S.*, **43**, 705 (1957)
37. Bammer, H., *Z. Zellforsch. u. mikroskop. Anat.*, **44**, 175 (1956)
38. Bazemore, A. W., Elliott, K. A. C., and Florey, E., *J. Neurochem.*, **1**, 334 (1957)
39. Bélanger, L. F., *Trans. Am. Otol. Soc.*, **44**, 94 (1956)
40. Belkering, D. H., Kuiper, J., and van Leeuwen, N. S., *Arch. Physiol. et Pharmacol. Neerl.*, **6**, 632 (1957)
41. Bell, R. P., *Endeavour*, **17**, 931 (1958)
42. Bennett, H. S., *J. Biophys. Biochem. Cytol.*, **2**, Suppl., 99 (1956)
43. Berger, M., *Biochim. et Biophys. Acta*, **23**, 505 (1957)
44. Boehm, G., *Kolloid. Zhur.*, **62**, 22 (1933)
45. Bornschein, H., *Experientia*, **14**, 13 (1958)
46. Bowsher, D., *Arch. Neurol. Psychiat.*, **78**, 377 (1957)
47. Bremer, F., and Stoupel, N., *Acta Physiol. et Pharmacol. Neerl.*, **6**, 487 (1957)
48. Brockman, J. A., Jr., and Burson, S. L., Jr., *Proc. Soc. Exptl. Biol. Med.*, **94**, 450 (1957)
49. Bruce, A. K., *J. Gen. Physiol.*, **41**, 693 (1958)
50. Bryant, S. H., and Tobias, J. M., *J. Cellular Comp. Physiol.*, **46**, 71 (1955)
51. Bullock, T. H., and Fox, W., *Quart. J. Microscop. Sci.*, **98**, 219 (1957)
52. Bullock, T. H., and Hagiwara, S., *Biol. Bull.*, **109**, 341 (1955)
53. Bullock, T. H., and Hagiwara, S., *J. Gen. Physiol.*, **40**, 565 (1957)
54. Burešová, O., *Physiol. Bohemosloven.*, **6**, 1 (1957)
55. Burgen, A. S. V., *Can. J. Biochem. and Physiol.*, **35**, 569 (1957)
56. Burns, D. D., *Can. J. Biochem. and Physiol.*, **34**, 380 (1956)

57. Caesar, R., Edwards, G. A., and Ruska, H., *J. Biophys. Biochem. Cytol.*, **3**, 867 (1957)
58. Caldwell, P. C., *J. Physiol. (London)*, **132**, 85P (1956)
59. Caldwell, P. C., and Keynes, R. D., *J. Physiol. (London)*, **137**, 12P (1957)
60. Cerf, J., *Arch. intern. pharmacodynamie.*, **109**, 300 (1957)
61. Chou, J. T. Y., *Quart. J. Microscop. Sci.*, **98**, 291 (1957)
62. Christensen, H. N., Riggs, R. T., Fischer, H., and Palatine, I. M., *J. Biol. Chem.*, **198**, 1, 17 (1952)
63. Churchill, J. A., Schuknecht, H. F., and Doran, R., *Laryngoscope*, **66**, 1 (1956)
64. Clemente, C. D., Sutin, J., and Silverstone, J. T., *Am. J. Physiol.*, **188**, 193 (1957)
65. Coelho, R. R., *J. Cellular Comp. Physiol.*, **49**, 261 (1957)
66. Coombs, J. S., Curtis, D. R., and Eccles, J. C., *J. Physiol. (London)*, **139**, 198, 232 (1957)
67. Cooper, S., and Daniel, P. M., *J. Physiol. (London)*, **42**, 222 (1957)
68. Cowie, D. B., and Roberts, R. B., in *Electrolytes in Biological Systems* (Shanes, A. M., Ed., American Physiological Society, Washington, D. C., 243 pp., 1955)
69. Cragg, B. G., and Thomas, P. K., *J. Physiol. (London)*, **136**, 606 (1957)
70. Crain, S. M., *J. Comp. Neurol.*, **104**, 285 (1956)
71. Csapo, A., and Suzuki, T., *J. Gen. Physiol.*, **41**, 1083 (1958)
72. Dalton, J. C., *J. Gen. Physiol.*, **41**, 529 (1958)
73. Davis, H., in *Physiological Triggers* (Bullock, T. H., Ed., American Physiological Society, Washington, D. C., 179 pp., 1957)
74. Deck, K. A., *Arch. ges. Physiol.*, **266**, 249 (1958)
75. Deitch, A. D., and Moses, M. J., *J. Biophys. Biochem. Cytol.*, **3**, 449 (1957)
76. Del Castillo, J., and Katz, B., *Proc. Roy. Soc. (London)*, [B]**146**, 339 et seq. (1957)
77. Del Castillo, J., and Suckling, E. E., *Federation Proc.*, **16**, 29 (1957)
78. De Lorenzo, A. J., *J. Biophys. Biochem. Cytol.*, **3**, 839 (1957)
79. De Lorenzo, A. J., *J. Biophys. Biochem. Cytol.*, **4**, 143 (1958)
80. De N6, R. Lorente, Vidal, F., and Larramendi, L. M. H., *Nature*, **179**, 737 (1957)
81. De Robertis, E., and Franchi, C. M., *J. Biophys. Biochem. Cytol.*, **2**, 307 (1956)
82. De Robertis, E., and Vaz Ferreira, A., *J. Biophys. Biochem. Cytol.*, **3**, 611 (1957)
83. Diamond, J., *J. Physiol. (London)*, **130**, 515 (1955)
84. Diamond, J., *J. Physiol. (London)*, **140**, 51P (1958)
85. Douglas, W. W., and Ritchie, J. M., *J. Physiol. (London)*, **138**, 31 (1957); **139**, 385 (1957)
86. Downing, A. C., and Wilkie, D. R., *J. Sci. Instr.*, **34**, 353 (1957)
87. Dybing, F., *Acta Pharmacol. Toxicol.*, **13**, 80 (1957)
88. Eccles, J. C., *Neurophysiological Basis of Mind* (Clarendon Press, Oxford, Engl., 314 pp., 1953)
89. Eccles, J. C., and Jaeger, J. C., *Proc. Roy. Soc. (London)*, [B]**148**, 38 (1958)
90. Eckel, R. E., Pope, C. E., II, and Norris, J. E. C., *Arch. Biochem. Biophys.*, **52**, 293 (1954)
91. Edgar, G. F. W., *Acta Anat.*, **31**, 451 (1957)
92. Edstr6m, J.-E., *J. Comp. Neurol.*, **107**, 295 (1957)
93. Edwards, C., and Kuffler, S. W., *Federation Proc.*, **16**, 34 (1957)

94. Edwards, G. A., Ruska, H., and DeHarven, E., *J. Biophys. Biochem. Cytol.*, **4**, 107 (1958)
95. Eggen, D., *Some Ultraviolet Microspectrophotometric Measurements on Isolated Nerve Axons* (Doctoral thesis, University of Chicago, Chicago, Ill., 1957)
96. Elliott, K. A. C., and Florey, E., *J. Neurochem.*, **1**, 181 (1956)
97. Fenn, W. O., *Proc. Soc. Exptl. Biol. Med.*, **96**, 783 (1957)
98. Fernández-Morán, H., and Finean, J. B., *J. Biophys. Biochem. Cytol.*, **3**, 725 (1957)
99. Fernández, C., and Brenman, A., *Am. J. Physiol.*, **188**, 249 (1957)
100. Finean, J. B., *J. Biophys. Biochem. Cytol.*, **3**, 95 (1957)
101. Finean, J. B., Hawthorne, J. M., and Patterson, J. D. E., *J. Neurochem.*, **1**, 256 (1957)
102. Finean, J. B., and Millington, P. F., *J. Biophys. Biochem. Cytol.*, **3**, 89 (1957)
103. Florey, E., *Can. J. Biochem. and Physiol.*, **34**, 669 (1956)
104. Florey, E., *J. Gen. Physiol.*, **40**, 553 (1957)
105. Folch, J., in *Psychiatric Research* (Harv. Univ. Monogs. in Med. & Pub. Hlth., No. 9, Harvard University Press, Cambridge, Mass., 1947)
106. Frankenhaeuser, B., *J. Physiol. (London)*, **137**, 245 (1957)
107. Frankenhaeuser, B., and Hodgkin, A. L., *J. Physiol. (London)*, **218**, 137 (1957)
108. Frankenhaeuser, B., and Persson, A., *Acta Physiol. Scand.*, **42**, Suppl. 145, 45, (1957)
109. Fry, W. J., *Neurology*, **6**, 693 (1956)
110. Fuortes, M. G. F., Frank, K., and Becker, M. C., *J. Gen. Physiol.*, **40**, 735 (1957)
111. Furukawa, T., *Japan. J. Physiol.*, **7**, 199 (1957)
112. Furukawa, T., Takagi, T., and Sugihara, T., *Japan. J. Physiol.*, **6**, 98 (1956)
113. Gamble, J. L., Jr., *J. Biol. Chem.*, **228**, 955 (1957)
- 113a. Gasser, H. S., *J. Gen. Physiol.*, **41**, 613 (1958)
114. Geren, B. B., and Schmitt, F. O., *Proc. Natl. Acad. Sci. U. S.*, **40**, 863 (1954)
115. Giacobini, E., *Acta Physiol. Scand.*, **42**, Suppl. 145, 49 (1957)
116. Giese, A. C., *J. Cellular Comp. Physiol.*, **49**, 295 (1957)
117. Glynn, I. M., *Progr. in Biophys. and Biophys. Chem.*, **8**, 241 (1957)
118. Goldsmith, T. H., and Philpott, D. E., *J. Biophys. Biochem. Cytol.*, **3**, 429 (1957)
119. Goodhead, P., *Acta Anat.*, **29**, 297 (1957)
120. Gray, J. A. B., and Inman, D. R., *J. Physiol. (London)*, **138**, 31P (1957)
121. Grundfest, H., *Acad. Sci. Georg. S.S.R.*, **21** (1956)
122. Grundfest, H., and Purpura, D. P., *Nature*, **178**, 416 (1956)
123. Hagiwara, S., and Bullock, T. H., *J. Cellular Comp. Physiol.*, **50**, 25 (1957)
124. Handovsky, H., *Kolloid. Zhur.*, **62**, 21 (1933)
125. Hara, T., *J. Gen. Physiol.*, **41**, 857 (1958)
126. Harris, E. J., and Steinbach, H. B., *J. Physiol. (London)*, **133**, 385 (1956)
127. Hashimura, S., and Wright, E. B., *J. Neurophysiol.*, **21**, 24 (1958)
128. Hild, W., *Z. Zellforsch. u. mikroskop. Anat.*, **47**, 127 (1957)
129. Hisada, M., *J. Cellular Comp. Physiol.*, **50**, 57 (1957)
130. Hodgkin, A. L., and Keynes, R. D., *J. Physiol. (London)*, **119**, 513 (1953)
131. Hodgkin, A. L., and Keynes, R. D., *J. Physiol. (London)*, **131**, 592 (1956)
132. Hodgkin, A. L., and Keynes, R. D., *J. Physiol. (London)*, **138**, 253 (1957)
133. Hubbard, S. J., *J. Physiol. (London)*, **137**, 40P (1957)
134. Hurlbut, W. P., *J. Gen. Physiol.*, **41**, 959 (1958)

135. Ichioka, M., *Japan. J. Physiol.*, **7**, 20 (1957)
136. Ichioka, M., and Konishi, K., *Japan. J. Physiol.*, **7**, 12 (1957)
137. Iggo, A., *Quart. J. Exptl. Biol.*, **42**, 130 (1957)
138. Issidorides, M., *Exptl. Cell. Research*, **11**, 423 (1956)
139. Ito, F., *Japan. J. Physiol.*, **7**, 86 (1957)
140. Ito, M., *Japan. J. Physiol.*, **7**, 297 (1957)
141. Itoh, S., and Schwartz, I. L., *Am. J. Physiol.*, **188**, 490 (1957)
142. Kao, C. Y., *J. Gen. Physiol.*, **40**, 107 (1956)
143. Kao, C. Y., and Grundfest, H., *Experientia*, **13**, 140 (1957)
144. Kao, C. Y., and Grundfest, H., *J. Neurophysiol.*, **20**, 553 (1957)
145. Katz, B., and Thesleff, S., *J. Physiol. (London)*, **138**, 63 (1957)
146. Kautz, J., and DeMarsh, Q. B., *Exptl. Cell. Research*, **8**, 394 (1955)
147. Kayushin, L. P., and Lyudkovskaya, R. G., *Proc. Acad. Sci. U.S.S.R.*, **95**, 253 (1954)
148. Kayushin, L. P., and Lyudkovskaya, R. G., *Proc. Acad. Sci. U.S.S.R.*, **102**, 727 (1955)
149. Kayushin, L. P., Lyudkovskaya, R. G., and Multanovsky, M. M., *Biophysics U.S.S.R.*, **1**, 405 (1956)
150. Kishimoto, U., *J. Gen. Physiol.*, **40**, 663 (1957)
151. Kirk, S. E., and Falk, G., *Experientia*, **14**, 36 (1958)
152. Köhler, W., and O'Connell, D. N., *J. Cellular Comp. Physiol.*, **49**, Suppl. 2, 1-43, (1957)
153. Koketsu, K., Cerf, J. A., and Nishi, S., *Federation Proc.*, **17**, 89 (1958)
154. Konishi, K., *Japan. J. Physiol.*, **7**, 1 (1957)
155. Konorski, J., and Lubinska, L., *Lancet*, **I**, 609 (1946)
156. Kornakova, E. V., and Frank, J. M., *Proc. Acad. Sci. U.S.S.R.*, **87**, 555 (1952)
157. Kornakova, E. V., Frank, J. M., and Steinhaus, L. N., *Fiziol. Zhur. S.S.S.R.*, **33**, 483 (1957)
158. Krnjević, K., *J. Physiol. (London)*, **135**, 281 (1957)
159. Kuffler, S. W., Fitzhugh, R., and Barlow, H. B., *J. Gen. Physiol.*, **40**, 683 (1957)
160. Kuno, M., *Japan. J. Physiol.*, **7**, 42 (1957)
161. Kunz, H. A., *Helv. Physiol. et Pharmacol. Acta*, **14**, 411 (1956)
162. Larramendi, L. M. H., Lorente de N6, R., and Vidal, F., *Nature*, **178**, 346 (1957)
163. Li, C. L., and McIlwain, H., *J. Physiol. (London)*, **139**, 178 (1957)
164. Liley, A. W., *J. Physiol. (London)*, **136**, 595 (1957)
165. Ling, G., *Am. J. Phys. Med.*, **35**, 1 (1955)
166. Ling, G., *Federation Proc.*, **17**, 98 (1958)
167. Ling, G., and Gerard, R. W., *J. Cellular Comp. Physiol.*, **34**, 413 (1949)
168. Lissak, K., and Endroczi, E., *Acta Physiol. Acad. Sci. Hung.*, **9**, 111 (1956)
169. Loewenstein, W. R., *J. Physiol. (London)*, **133**, 588 (1956)
170. Loewenstein, W. R., *J. Gen. Physiol.*, **41**, 825, 847 (1958)
171. Loewenstein, W. R., and Altamirano-Orrego, R., *J. Gen. Physiol.*, **41**, 805 (1958)
172. Loewenstein, W. R., and Rathkamp, R., *Science*, **127**, 341 (1958)
173. Lowenstein, O., *Proc. Roy. Soc. Med.*, **45**, 133 (1952)
174. Lubinska, L., *Exptl. Cell Research*, **10**, 40 (1956)
175. Lucas, D. R., and Newhouse, J. P., *Arch. Ophthalmol.*, **58**, 193 (1957)
176. Luse, S. A., *J. Biophys. Biochem. Cytol.*, **2**, 777 (1956)
177. Luxoro, M., *Proc. Natl. Acad. Sci. U. S.*, **44**, 152 (1958)

178. Lyall, A. H., *Quart. J. Microscop. Sci.*, **98**, 189 (1957)
179. Malhotra, S. K., *Quart. J. Microscop. Sci.*, **98**, 65 (1957)
180. Maxfield, M., *J. Gen. Physiol.*, **37**, 201 (1953)
181. Maxfield, M., and Hartley, R. W., *J. Biophys. Biochem. Cytol.*, **1**, 279 (1955)
182. Maxfield, M., and Hartley, R. W., *Biochim. et Biophys. Acta*, **24**, 83 (1957)
183. McIntyre, A. R., *Curare* (University of Chicago Press, Chicago, Ill., 240 pp., 1947)
184. McLennan, H., *J. Physiol. (London)*, **139**, 79 (1957)
185. Mercer, E. H., *Nature*, **180**, 87 (1957)
186. Miller, W. H., *J. Biophys. Biochem. Cytol.*, **3**, 421 (1957)
187. Mizukoshi, O., Konishi, T., and Nakamura, F., *Ann. Otol. Rhinol. Laryngol.*, **66**, 106 (1957)
188. Motokawa, K., Oikawa, T., and Tasaki, K., *J. Neurophysiol.*, **20**, 186 (1957)
189. Müller, P., in *Internationales Symposium über den Mechanismus der Erregung* (Deut. Verlag. der Wissen., Berlin, Germany, 1956)
190. Mullins, L. J., in *Molecular Structure and Functional Activity of Nerve Cells* (Waverly Press, Baltimore, Md., 169 pp., 1956)
191. Nakai, J., *Am. J. Anat.*, **99**, 81 (1956)
192. Nasonov, D. N., and Suzdal'skaia, I. P., *Fiziol. Zhur. S.S.S.R.*, **43**, 617 (1957)
193. Nelson, P. G., *J. Cellular Comp. Physiol.*
194. Nevis, A. H., *J. Gen. Physiol.*, **41**, 927 (1958)
195. Noell, W. K., *U.S.A.F. School of Aviation. Med., Rept. 1, Proj. No. 21-1201-0004*, Randolph Field, Texas, Oct., (1953)
196. Paintal, A. S., *J. Physiol. (London)*, **135**, 486 (1957)
197. Pantin, C. F. A., *J. Exptl. Biol.*, **13**, 159 (1936)
198. Pappius, H. M., and Elliott, K. A. C., *Can. J. Biochem. and Physiol.*, **34**, 1053 (1956)
199. Paton, H. D., *Ann. Rev. Physiol.*, **20**, 509 (1958)
200. Pease, D. C., and Quilliam, T. A., *J. Biophys. Biochem. Cytol.*, **3**, 331 (1957)
201. Pierce, S., and Giese, A. C., *J. Cellular Comp. Physiol.*, **49**, 303 (1957)
202. Post, R. L., and Jolly, P. C., *Biochim. et Biophys. Acta*, **25**, 118 (1957)
203. Punt, A., Nijhof-Rombach, F., and Schippers, B., *Acta Physiol. et Pharmacol. Neerl.*, **6**, 551 (1957)
204. Purpura, D. P., Girado, M., and Grundfest, H., *Science*, **125**, 1200 (1957)
205. Purpura, D. P., Girado, M., and Grundfest, H., *Proc. Soc. Exptl. Biol. Med.*, **95**, 791 (1957)
206. Quilliam, T. A., *J. Physiol. (London)*, **137**, 2P (1957)
207. Rahman, M. H., Frazier, L. E., Hughes, R. H., and Cannon, P. R., *Arch. Path.*, **63**, 154 (1957)
208. Reger, J. F., *Exptl. Cell Research*, **12**, 662 (1957)
209. Relman, A. S., *Yale J. Biol. Med.*, **29**, 248 (1956)
210. Rideal, E., and Taylor, F. H., *Proc. Roy. Soc. (London)*, [B] **148**, 450 (1958)
211. Ries, H. E., and Kimball, W. A., *Nature*, **181**, 901 (1958)
212. Ries, H. E., and Kimball, W. A., in *Proc. Second Intern. Conf. Surface Act'y.*, **1**, 75 (Schulman, J. H., Ed., Butterworth Scientific Publications, London, Engl., 1957)
213. Ritchie, J. M., and Straub, R. W., *J. Physiol. (London)*, **136**, 80 (1957)
214. Roberts, E., Rothstein, M., and Baxter, C. F., *Proc. Soc. Exptl. Biol. Med.*, **97**, 796 (1958)
215. Robertson, J. D., *J. Biophys. Biochem. Cytol.*, **2**, 381 (1956)

216. Robertson, J. D., *J. Biophys. Biochem. Cytol.*, **3**, 1043 (1957)
217. Robertson, J. D., *J. Physiol. (London)*, **137**, 8P (1957)
218. Robertson, J. D., *J. Biophys. Biochem. Cytol.*, **4**, 39 (1958)
219. Robertson, J. D., *J. Physiol. (London)*, **140**, 58P (1958)
220. Rossiter, R. J., McLeod, I. M., and Strickland, K. P., *Can. J. Biochem. and Physiol.*, **35**, 945 (1957)
221. Schlote, F. W., *Z. Zellforsch. u. mikroskop. Anat.*, **45**, 543 (1957)
222. Schmidt, H., and Stämpfli, R., *Helv. Physiol. et Pharmacol. Acta*, **15**, 200 (1957)
223. Schmitt, F. O., *J. Cellular Comp. Physiol.*, **49**, Suppl. 1, 165 (1957)
224. Schoffeniels, E., *Science*, **124**, 1117 (1958)
225. Schoffeniels, E., *Biochim. et Biophys. Acta*, **27**, 660 (1958)
226. Schoffeniels, E., and Nachmansohn, D., *Biochim. et Biophys. Acta*, **26**, 1 (1957)
227. Schultz, R. L., Maynard, E. A., and Pease, D. C., *Am. J. Anat.*, **100**, 369 (1957)
228. Segal, J., *Physiol. Bohemosloven.*, **6**, 285 (1957)
229. Shaw, F. H., Simon, S. E., Johnstone, B. M., and Holman, M. E., *J. Gen. Physiol.*, **40**, 1, 263 (1956)
230. Shelley, W. B., and Arthur, R. P., *Arch. Dermatol.*, **76**, 296 (1957)
231. Shen, S. G., Greenfield, P., and Boell, E. J., *J. Comp. Neurol.*, **106**, 433 (1956)
232. Sheveleva, V. S., *Doklady Akad. Nauk. S.S.S.R.*, **112**, 148 (1957)
233. Simon, S. E., Shaw, F. H., Bennett, S., and Muller, M., *J. Gen. Physiol.*, **40**, 753 (1957)
234. Sjöstrand, F. S., *Intern. Rev. Cytol.*, **5**, 455 (1956)
235. Skou, J. C., *Biochem. et Biophys. Acta*, **23**, 394 (1957)
236. Smith, C. A., *Trans. Am. Otol. Soc.*, **44**, 23 (1956)
237. Smith, C. A., and Dempsey, E. W., *Am. J. Anat.*, **100**, 337 (1957)
238. Solomon, A. K., Lionetti, F., and Curran, P. F., *Nature*, **178**, 582 (1956)
239. Solomon, S., and Tobias, J. M., *Biol. Bull.*, **101**, 198 (1951)
240. Spyropoulos, C. S., *J. Gen. Physiol.*, **40**, 849 (1957)
241. Spyropoulos, C. S., *Am. J. Physiol.*, **189**, 215 (1957)
242. Stämpfli, R., and Nishie, K., *Helv. Physiol. et Pharmacol. Acta*, **14**, 93 (1956)
243. Stämpfli, R., and Willi, M., *Experientia*, **13**, 297 (1957)
244. Steinbach, H. B., *Am. J. Physiol.*, **163**, 236 (1950)
245. Stephenson, W. K., *J. Cellular Comp. Physiol.*, **50**, 105 (1957)
246. Sulkin, N. M., *J. Biophys. Biochem. Cytol.*, **1**, 459 (1955)
247. Svaetichin, G., *Acta Physiol. Scand.*, **39**, Suppl. 134, 1-112, (1956)
248. Szent-Györgyi, A., *Bioenergetics* (Academic Press, Inc., New York, N. Y., 143 pp., 1957)
249. Tasaki, I., *Am. J. Physiol.*, **190**, 575 (1957)
250. Tasaki, I., and Bak, A., *Science*, **126**, 696 (1957)
251. Tasaki, I., and Bak, A., *J. Neurophysiol.*, **21**, 124 (1958)
252. Tasaki, I., and Hagiwara, S., *J. Gen. Physiol.*, **40**, 859 (1957)
253. Terzuolo, C. A., and Bullock, T. H., *Proc. Natl. Acad. Sci. U. S.*, **42**, 687 (1956)
254. Thiel, W., *Acta Anat.*, **31**, 156 (1957)
255. Thies, R. E., *Biol. Bull.*, **113**, 333 (1957)
256. Tobias, J. M., *J. Cellular Comp. Physiol.*, **36**, 1 (1950)
257. Tobias, J. M., in *Modern Trends in Physiology and Biochemistry* (Barron, E. S. G., Ed., Academic Press, Inc., New York, N. Y., 538 pp., 1952)
258. Tobias, J. M., *Cold Spring Harbor Symposia Quant. Biol.*, **17**, 15 (1952)
259. Tobias, J. M., *J. Cellular Comp. Physiol.*, **52**, 89 (1958)
260. Tomita, T., *Japan J. Physiol.*, **7**, 80 (1957)

271. Tomita, T., *J. Neurophysiol.*, **20**, 245 (1957)
271a. Tosteson, D. C., *J. Cellular Comp. Physiol.*, **50**, 199 (1957)
272. Trendelenburg, U., *J. Physiol. (London)*, **135**, 66 (1957)
273. Ungar, G., Ascheim, E., Psychoyos, S., and Romano, D. V., *J. Gen. Physiol.*, **40**, 635 (1957)
274. Ungar, G., and Romano, D. V., *Proc. Soc. Exptl. Biol. Med.*, **97**, 324 (1958)
275. Uzman, B. G., and Nogueira-Graf, G., *J. Biophys. Biochem. Cytol.*, **3**, 589 (1957)
275a. Van der Kloot, W. G., *J. Gen. Physiol.*, **41**, 879 (1958)
276. van Harreveld, A., and Christensen, E., *Acta Bhysiol. et Pharmacol. Neerl.*, **6**, 597 (1957)
277. van Harreveld, A., and Ochs, S., *Am. J. Physiol.*, **189**, 159 (1957)
278. Vodička, Z., *Physiol. Bohemosloven*, **5**, Suppl., 55 (1956)
279. Wasano, T., Ogata, M., and Goto, M., *Japan J. Physiol.*, **6**, 137 (1956)
280. Welkowitz, W., and Fry, W. J., *J. Cellular Comp. Physiol.*, **48**, 435 (1956)
281. Wersäll, J., *Acta Oto-Laryngol.*, Suppl., 126 (1956)
282. Whitear, M., *Experientia*, **13**, 287 (1957)
283. Whiting, H. P., *Quart. J. Microscop. Sci.*, **98**, 163 (1957)
284. Wolken, J. J., Capenos, J., and Turano, A., *J. Biophys. Biochem. Cytol.*, **3**, 441 (1957)
285. Wood, D. W., *J. Physiol. (London)*, **138**, 119 (1957)
286. Yamasaki, T., and Narahashi, T., *Botyu-Kagaku* (English), **22**, 259 (1957)
287. Yamasaki, T., and Ishii, T., *Publ. Inst. Insect Control*, 163 (Kyoto Univ., World Health Org., Oct. 1957)
288. Zacharova, D., *Physiol. Bohemosloven.*, **6**, 143, 353, 360 (1957)
289. Zhirmunsky, A. V., *Doklady Akad. Nauk. U.S.S.R.*, **112**, 157 (1957)

SOMATIC FUNCTIONS OF THE NERVOUS SYSTEM¹

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AFFERENT SYSTEMS

Vision.—Using the technique developed by Talbot & Kuffler (183, 297), Barlow *et al.* have demonstrated an increase in threshold in the light-adapted eye when the size of the light spot exceeds a certain magnitude (14). This change is interpreted as being caused by inhibition on the central parts of the receptive field exerted by surrounding areas. In the dark-adapted retina, there is no antagonistic interaction between peripheral and central parts of the receptive field. During dark adaptation, the lateral inhibition gradually ceases and summation occurs when the stimulus spot is increased. In this connection, the beautifully performed investigations by Hartline and co-workers on the *Limulus* eye are of great interest (148, 149). Here mutual interaction occurs at the receptor level and is conveyed by the fibre plexus behind the ommatidia. Although this interaction is purely inhibitory and is mediated without participation of internuncial neurons, it produces complex effects, as illustrated by the fact that disinhibition may simulate facilitation. Brindley has provided evidence that the electroretinogram (ERG) is generated in the receptors [see (41, 42)]. Further, by using small test spots he has demonstrated that no interaction occurs between different parts of the retina producing the ERG (43), suggesting that no significant part of the ERG arises in the neural cell layers. This view is further strengthened by the finding that the complex intraretinal potentials described by Tomita and others may be absent although the ERG is normal. In other studies on potentials, a separate direct current component was obtained in isolation in the light-adapted eye of the cat (49).

There seems still to be a great deal of confusion as to the origin of the sustained intraretinal potential designated as cone action potential (293). Similar potentials have been found in the eyes of different fishes (249) and in the cat's retina (142). According to Tomita (299), these potentials in the fish eye do not arise in the receptors. It would be possible to settle the question about the origin of these responses if the tip of the electrode could be accurately located within the retina. Recent attempts in this direction indicate that these sustained potentials arise in structures proximal to the receptors (224). The human flicker ERG has been studied (150), and it is suggested that the splitting of the b-wave and the off-effect result from colour components with different latencies. Particular features of the ERG in colour-blind persons have also been demonstrated (151). Other reports deal with the effect on the ERG of dazzling the eye (174), retinal responses to

¹ The survey of the literature pertaining to this review was concluded in April, 1958.

light stimuli of short duration (143, 144, 175), and spectral sensitivity of the turtle's (78) and the cat's eyes (88, 89).

Latency measurements of single unit responses in the optic tract to electrical stimulation of the optic nerve have revealed in cats three latency groups corresponding to average conduction velocities of 52, 37, and 16 mm. per sec. (195). No response from the large group of fibres with a diameter less than $1\ \mu$ could be obtained. According to Lennox (196), slow fibres respond more actively to blue than to red light. This fact would provide an adequate explanation of the shorter central transmission time (63, 194, 197) when the eye is illuminated with red rather than with blue light. A prolonged "second subnormality" of the lateral geniculate postsynaptic response after high-frequency tetanization of the optic nerve has been described and it is found that the degree of posttetanic potentiation of the response is related to the degree of subnormality present, there being no, or insignificant posttetanic potentiation in the absence of induced subnormality at this relay (114). The appearance of the long-lasting subnormality is dependent on the anesthetic state (115). Concerning the influence of anesthetics on the responses in the lateral geniculus and the striate cortex to photic stimulation, recent investigations show a progressive increase in latency with increasing depth of anesthesia (170).

The convergence towards cortical units via the specific and diffuse projection systems [see (55, 80, 81, 168, 198)] has been investigated within the visual cortex in *encéphale isolé* preparations (4, 74) to stimulation of the retina with light (19, 171 to 173), and to electrical stimulation of different thalamic regions (cf. 3, 73, 75, 76). All neurons studied, which responded to light, could also be activated from nonspecific thalamic regions. Different types of interaction between facilitation and inhibition were described.

Audition.—In records of the cochlear responses in the unanesthetized guinea pig (192, 193), considerable fluctuations in the amplitude of the microphonics as well as in the action potentials were observed. These variations were partly ascribed to contractions of the muscles of the middle ear and partly to the action of inhibitory fibres.

Previous studies (37, 68, 273) have established that binaural interaction occurs in the inferior colliculus and auditory cortex. Earlier attempts to demonstrate interaction at the lateral lemniscus have, however, failed. This problem has been reinvestigated (274), and a comparison of the responses recorded at the lateral meniscus to monaural and binaural stimuli shows that there is a statistically significant interaction similar to that observed in the inferior colliculus.

The recognition of the difference in reaction of anesthetized and unanesthetized animals has given rise to a series of investigations in which the technique for recording with implanted electrodes has been applied. Whitfield (307) has recorded the potentials in the auditory cortex in intact cats. Pure tone stimulation either evoked a fast activity or caused no change in the resting discharge. The regular oscillatory potentials described by Bremer (36, 38) were not observed. By modulating a tonal stimuli it was,

however, possible to elicit rhythmical waves and drive the cortical activity at a frequency corresponding to that of the modulated stimulus. In addition to the three portions (A_1 , A_2 , and E_p) of the ectosylvian auditory area and the area in the ventral anterior ectosylvian gyrus (A_3), a suprasylvian auditory area (SS) has been found in the cat responding to click stimuli (216). The persistence of the SS response after ectosylvian ablation indicates that it is not caused by spread from distant active regions. It is argued that the cochlea exerts a tonic inhibitory influence on the ipsilateral SS response, because it increases after cochlear destruction. Since the SS area corresponds to the field from which conjugate eye movements can be evoked, this area is considered to serve cortical reflexes for oriented alerting to sound.

Olfaction, taste, pain, thermoreception.—Olfaction still appears to be an unattractive subject to electrophysiologists, as revealed by the paucity of reports published in this field. Recent studies of the discharge pattern in the olfactory bulb (253) indicate that the spatial and temporal differentiation of responses to different odorous substances cannot be the adequate basis for olfactory discrimination.

Experiments on the goat with local thalamic stimulation and destruction have presented further evidence of the localization of the thalamic relay for taste in the posteromedialventral thalamic nucleus (8). The impulse patterns of cortical cells within the tongue projection area obtained in response to gustatory mechanical and thermal stimulation of the tongue (67, 189, 190) show that the majority of the cells were fired by one type of stimulus only, a smaller fraction being activated by different sensory modalities. Cortical taste cells responded to all types of gustatory stimulation tested. It is of interest to note that cortical touch cells within the tongue area could fire in a definite pattern which was the same whether the afferent path was activated electrically or physiologically.

The widely discussed problem of the central neural mechanisms serving pain [see (220)] has been studied in unanesthetized cats (233), in which two types of evoked cortical potentials in the ventral portion of the ectosylvian gyrus could be obtained in response to electrical tooth-pulp stimulation: one contralateral short-latency response unaffected by nitrous oxide and one bilateral long-latency response susceptible to the gas in a concentration which causes analgesia but is insufficient for anesthesia in man. In studies on the recovery after spinal cord injuries in humans (11), itch sensation was shown to be correlated with the preservation of pain but not with the presence of touch. These observations are in line with Zotterman's conclusion that itch is dependent on the small afferents (311), and Bishop's considerations concerning the physiological characteristics of itch (30).

Thermoreceptor activity has been shown to be influenced by the peripheral blood flow altered by stimulation of autonomic fibres, but no evidence was found for a direct efferent innervation of thermoreceptors (87).

Vestibular functions.—Evidence is presented that the ampullae of Lorenzini, like other organs of the acousticolateralis system, are sensitive to mechanical stimuli (255). However, they only respond to stimuli, which

cause a change in pressure within the ampullae relative to the pressure outside. This observation explains why the ampullae have earlier been considered as insensitive to mechanostimulation. Bekešy's discovery of the direct current potentials in the cochlea has directed attention to the functional importance of steady potentials in the labyrinth. Analogous potentials have been demonstrated in the semicircular canals (301); deviation of the cupula in the horizontal canal in the utriculopetal direction was found to give a negative potential change, while deviation in the opposite direction evoked a positivity. These observations are of particular interest in view of Loewenstein & Sand's classical finding that deviation of the cupula in one direction elicits an impulse discharge in the vestibular nerve, while deviation in the opposite direction inhibits the resting discharge, and Trincker suggests that the slow potential changes are to be considered as generator potentials.

New data concerning the connections and topography of the vestibular nuclei are presented (46, 48, 263). The anatomical structures of the different nuclei suggest that they operate in functionally different systems. The origin of the secondary vestibulocerebellar fibres is traced in studies on the retrograde degeneration following restricted lesions in the cerebellar cortex. It is further demonstrated that the fibres of the vestibulospinal tract have their origin in the lateral nucleus.

Stimulation of the vestibular nerve in the cat elicits a two-peak response in the lumbar ventral roots which disappears after ventral quadrant transection (127). The transmission time in the descending fibres was not measured but, according to the authors, the latency of the first volley indicates a monosynaptic activation of the spinal motoneurons, whereas the second response should be mediated via reticulospinal pathways.

The effects of labyrinthine stimulation and stimulation of the skin by touch and pressure on the motor discharge to cervical muscles have been studied by Nakayama (256). Reciprocal effects were demonstrated in ipsi- and contralateral muscles and it was further shown that the effect of pressure summated algebraically with that of tilting and rotation. The results give a detailed picture of the activity patterns in muscles taking part in reflexes which are designated as "body righting reflexes acting upon the head." A series of studies of vestibular influences on single unit activity in the medial part of *formatio reticularis* during different phases of vestibular nystagmus evoked by lesions in the vestibular nucleus has been made by Duensing & Schaefer (94, 95). They recorded reticular interneuron impulse patterns extracellularly and differentiated the neurons into two groups, the activity of the first of which was rigidly coupled to the nystagmus, that of the second loosely coupled. In both groups, neurons were found which were activated during the slow and fast phases of nystagmus. Reciprocal relations between neurons were also found. The discharge patterns of reticular units elicited by activation from cerebellar and the cerebral cortex have been described earlier (7, 239), as has their activation by different types of sensory stimulation (7, 16); and, according to Duensing and Schaefer, there is a diffuse convergence from the different sensory receptor fields into the inter-

neurons which are loosely coupled to nystagmus. The importance of the vestibular inflow into the ascending reticular system is stressed and the mechanism underlying the rhythmical activity of nystagmus is discussed in relation to earlier considerations by Lorente de Nó (217, 218).

Proprioception from muscles.—Among investigations on nerve impulse generation in proprioceptors may be mentioned experiments in which the potential of the lobster's stretch receptor in response to stretch and to antidromic stimulation of the axon was studied with microelectrodes placed under microscopic control on the cell body and at various places along the axon (60). It was shown that the impulse initiated by stretch starts in the axon near the cell body and from there propagates out along the axon as well as back into the cell body. Extracts from lobster nerve cords and nerves have been tested on the inhibitory dendrite synapses of the lobster's stretch receptor (110) and were found to produce inhibition or acceleration of the sensory discharge, or reduction in effectiveness of inhibitory transmission, depending on concentration and source of the extracts. Some extracts gave inhibitory effects of the same type as those of γ -aminobutyric acid. Eyzaguirre (117) presents an excellent survey of the sensory functions of the muscle spindle and the different properties of the crustacean, amphibian, and mammalian stretch receptors and presents a study on the sensory discharge and intrafusal activity in response to motor axon stimulation. Twitch fibres were demonstrated in the toad's spindles, which were found to have a multiple innervation both by small and large motor fibres, both of which were also shown to supply extrafusal fibres. The small efferents activate slow intrafusal fibres, and one intrafusal bundle may be innervated by large and small nerve fibres, which means that it has a double motor control. The junctional potential of intrafusal muscle fibres (i.j.p.) in the frog has been investigated by Koketsu & Nishi with intracellular electrodes (177, 178). The characteristics of the i.j.p. were described, and the action of acetylcholine on the intrafusal muscle membrane was found to be the same as on the twitch and slow muscle membrane.

In connection with the discussion of the efferent innervation of the mammalian muscle spindles, Liljestrand & Magnus' observation (203) that a local anesthetic applied to a muscle blocks the hypertonus from decerebration before affecting the muscle response to motor nerve stimulation has become of considerable interest since the demonstration of the functional significance of the gamma-efferents governing the muscle spindle activity. The effect of procaine locally applied to a muscle nerve on stretch reflexes in different types of preparations, on different fractions of the compound nerve action potential, and on the discharge of single muscle spindles has been tested in a series of clear-cut investigations (226, 227, 229 to 232) showing that the gamma-efferents are more sensitive to procaine than the large efferent and afferent fibres.

The nerve in the cat innervating the intrinsic laryngeal muscles has an unimodal fibre-size distribution with a sharp peak about 10 to 12 μ (254) and, according to unpublished observations (254), the intrinsic muscles of

the cat larynx have no spindles. The assumption (120) that muscles, which are supplied by fibres of intermediate size, unimodally distributed, always have a scanty or no proprioceptive supply remains an open question. As to the stretch receptors in mammalian eye muscles, the work by Whitteridge *et al.* (70, 71, 72, 308) has been continued by Cooper & Daniel (69), who conclude that there is no reason to believe that muscle spindles and tendon endings in the goat's eye muscles behave differently from those in the mammalian limb muscles.

The importance of the frequency modulation in spinal interneurons has been stressed in earlier studies on single unit activity (123, 186). Kolmodin & Skoglund (180) studied the convergence in spinal interneurons with intracellular microelectrodes and demonstrated the importance of spinal interneurons as convergence pools for different sensory modalities (104, 105). An extensive analysis has been made by Kolmodin (179) of the convergence to, and frequency modulation in, a large sample of spinal interneurons activated by stimulation of sense sensory end organs in the muscles. The relation between the in- and outgoing information has been expressed in frequency curves obtained with impulse recorders and counting rate meters. The majority of the interneurons studied gave maintained frequency responses when slowly adapting stretch receptors were activated. The relation between the frequencies of the afferent discharge and the interneuron discharge revealed signs of central adaptation. The interneurons tested could be differentiated into five different types with regard to the convergence patterns from different muscles, and 50 per cent of these interneurons could be activated from different exteroceptive origins. In the majority of cases, simultaneous stimulation of two excitatory afferents produced an increase of the interneuronal discharge. The discharge pattern in response to inhibitory and excitatory interaction was also studied, and the results indicate that in the interneuron pool there may appear patterns of activity which are unique for certain movements or positions of the limb.

Mechanoreceptors (skin).—Of basic importance are investigations on the physical mechanisms underlying the receptor potential in mechanoreceptors (157, 158), in which the relation between the external and internal mechanical changes in Pacinian corpuscles is studied. Radial displacement of the corpuscle lamella because of compression was measured and its physiological significance discussed. Concerning the electrical excitation processes in mechanoreceptors, the initiation of nerve impulses in Pacinian corpuscles has been further investigated (85, 86, 214, 215). Conduction and excitability characteristics of sensory axon terminations (62), as well as the integration of the confluent impulses in the branches of the peripheral nerve fibres in response to mechanical stimulation of separated touch endings in the frog (204), have also been studied.

As to the conduction of touch impulses in peripheral cutaneous nerves, Douglas & Ritchie (90, 91) have used their special "reduction technique" which allows a better evaluation than before of the activity in nonmedullated nerve fibres. They found (92) that in the cat saphenous nerve not only

the fastest and slowest medullated but also nonmedullated fibres are activated by touch and light pressure. Two subgroups of nonmedullated fibres are distinguished, fast and slow, the first being especially engaged in touch. The firing frequency in response to touch was found to be low, maximally 10 impulses per sec. (93).

De Molina & Gray recorded the negative spinal cord potential from the dorsal horn in response to electrical stimulation of low threshold cutaneous fibres, and differentiated it into two phases with different latencies (237). Both phases were ascribed to activity in postsynaptic neurons, the second one being compared with cord dorsum N_1 -deflection earlier described (23). Cutaneous and proprioceptive projection to the anterior sigmoid gyrus has been demonstrated on cats (259), and the somatic receiving areas I and II of the squirrel monkey have been mapped by recording the surface positive potentials elicited by mechanical skin stimulation (21). It is suggested that a part of the responsive cortex is coextensive with the precentral motor pattern. According to Livingston & Phillips (207), the maps of the tactile receiving area in the cat are largely coextensive with the motor maps in the same individual. In most investigations, the importance of taking into account the narcotic state is stressed, and further observations of the differential effects of pentobarbital and chloralose on cortical evoked responses have been presented (119).

The studies by Mountcastle *et al.* on the response properties of single units in the cat's somatic sensory cortex (251, 252) have led to interesting considerations concerning the pattern of functional organization in the first sensory projection area. The advantage of using natural stimulation in this special type of investigation is obvious. The importance of taking into account the functional state of the neurons as judged by such attributes as their susceptibility to being driven from peripheral sources, when considering the validity of the results, has been carefully discussed, by Kolmodin concerning intracellularly recorded activity from spinal interneurons (179) and by Mountcastle *et al.* concerning cortical neuron activity recorded with extracellular microelectrodes. Mountcastle *et al.* studied the firing patterns of single units within the sensory cortex in response to different types of light mechanical skin stimulation in anesthetized cats. The experimental data serve as a basis for an interesting discussion of the mechanisms of sensory localization, two-point discrimination, and contour recognition. The characteristics of the discharge patterns of the cortical cells with regard to latency, frequency, and number of impulses occurring most frequently in the population of responses were analysed in relation to the type of the peripheral stimulus. Under the circumstances given, three types of cells could be differentiated with regard to the mode of activation (movements of hair, pressure upon skin, deformation of deep tissues). The neurons belonging to each of these groups also showed differentiated discharge patterns. The neurons belonging to each group were found to be intermingled in all the cortical cell layers and organized in perpendicular columns which correspond to distinct peripheral receptive fields. The results thus point to the

existence of elementary functional units which are capable of complex in- and output functions independent of horizontal spread. This arrangement is in line with assumptions first made by Lorente de Nó (219) on the basis of histological data and later on by Sperry (284, 285) on the basis of observations on the persistence of higher central functions after vertical dicing of the cortex. The spatial extent of the electrical field surrounding one active cortical neuron was also mapped. Its extent, compared with the degree of interlocking of the dendritic fields, makes ephaptic interaction between the neurons possible. Other investigations of the time interrelationship between the impulse discharges of neighboring single cortical neurons also point to the existence of an ephaptic interaction (17, 18).

SPINAL REFLEX ACTIVITY

Opinions about the mechanisms of short latency inhibitory spinal reflex action have been conflicting since the original suggestion of the possibility of direct inhibitory effects on motor neurons from large muscle afferents was made (209). The argument has centered around the question of the existence of an intercalated neuron in the inhibitory pathway. If this be true, a difference should be demonstrable in the central delay of inhibitory and excitatory influence on the motoneurons induced by stimulation of the muscle spindle afferents (Group Ia muscle afferents) in the myotatic unit. According to Eccles *et al.* (105), the latency of the intracellularly recorded hyperpolarization of the motor neuron membrane (IPSP) in response to inhibitory volleys is shorter than that of the excitatory postsynaptic potential (EPSP), a fact leading to the postulation that there is an interneuron in the inhibitory pathway. Later observations on the difference in threshold for the two synaptic actions and the summation of IPSP (106), as well as on the growth of facilitation and inhibition with increasing afferent volleys (167), are also taken as evidence for an interneuron in the inhibitory pathway. The former findings (106) are interpreted in relation to the hypothesis that central synaptic actions are mediated by chemical transmitters.

A quantitative evaluation has been made of the receptor fields for monosynaptically excited motoneurons in the cervical and lumbar spinal cord in the cat by intracellular recording of the responses from a large sample of motoneurons to electrical stimulation of different nerves (100). In general, the conclusion is confirmed that the receptor field is largely located in the muscle innervated by the motoneuron in question (homosynaptic influence) and in muscles serving as synergists in the same joint (210, 211). However, monosynaptic influences on motoneurons were also found from afferents originating in muscles which are not to be regarded as synergists. The importance for determination of reflex excitability of taking into account the fact that certain motoneurons fail to respond to homosynaptic stimulation at intervals shorter than 3 sec. is stressed by studies showing a differential behaviour of the facilitatory and inhibitory components in the myotatic reflex system with repetitive stimulation at low frequencies (29). Group I afferents from muscles may be divided functionally into two groups with regard to

slight differences in threshold and conduction velocity (99); fibres with faster conduction are identical with the afferents from annulospinal endings (Group Ia afferents), and slow conducting fibres are identical with Golgi tendon organ afferents (Group Ib afferents), there being a higher degree of differentiation with regard to threshold than with regard to conduction velocity. Concerning the segmental reflex action of Golgi tendon organ afferents (Ib afferents), recent investigations (101) confirm earlier findings (191) that their excitatory and inhibitory action on the motoneurons is disynaptically mediated. However, they also demonstrate a trisynaptic conduction for both excitatory and inhibitory actions. Extensor muscle motoneurons frequently receive inhibitory Ib actions from extensors and rarely from flexors. The Ib excitatory action on flexor muscle motoneurons, on the other hand, has a widespread origin, being located not only in antagonists in extensor muscles but also in extensor and flexor muscles of other joints.

Evidence has earlier been presented that the ventral motor horn cells in the cat, innervating extrafusal muscle fibres (alpha motoneurons) and giving tonic and phasic activity, can be differentiated functionally, e.g., by posttetanic potentiation (133). According to later investigations (138) of the differential behaviour of small and large spikes in the ventral root filaments to different types of activation, "tonic" motor horn cells tend to be of a smaller dimension than "phasic", a conclusion earlier reached on the basis of ischemia experiments (165). The observations (102) that alpha motoneurons with a relatively slow conduction velocity preferentially innervate tonic muscles, as defined by Denny-Brown (82), and the fact that the slowly conducting alpha motoneurons have a longer afterhyperpolarization than the rapidly conducting motoneurons provide interesting evidence as to their different functional characteristics. It has been claimed that the Renshaw cells are monitored by the recurrent motoneuron collaterals, that the recurrent circuit mediates inhibition (103), and further that recurrent collaterals serve to stabilize the motoneuron discharge (145, 155). Recently, the importance of this feedback system for the "tonic" alpha cell function as an inhibitory stabilizing mechanism has been stressed (134).

Reinvestigation of the cause of posttetanic potentiation in the spinal monosynaptic reflex arc by microelectrode stimulation in different parts of the lumbar enlargement (305) confirms earlier conclusions concerning its presynaptic origin (212), and evidence is presented that the posttetanic change is greater in the terminal arborizations than in other parts of the afferent axon.

Lloyd has, in addition to his earlier studies on the monosynaptic reflex pathway, analysed the input-output relation in a flexor reflex arc activated by stimulation of cutaneous group II fibres (213). From threshold to maximal input, the relation between input and amount of output was essentially linear. On the average, the latency of the discharge decreased with increasing input but the minimum pathway of three neurons was not found to transmit, unless aided by convergent activity.

Data are presented on the influence on hind leg motoneurons from af-

ferents of the opposite hind leg (261, 262) as well as from visceral afferents (113). Electrical stimulation of low threshold cutaneous fibres (14 to 16 μ) facilitates crossed knee- and ankle-flexor motoneurons after a central delay of 3 msec. and may even cause a discharge crossed extensor motoneurons often being simultaneously depressed. Excitation of small myelinated cutaneous afferents (6 to 2 μ) produces prolonged inhibition of crossed flexor motoneurons and a long-lasting facilitation of extensor motoneurons after a central delay of 6 to 30 msec. Stimulation of Group I muscle afferents evokes transient inhibition and subsequent facilitation of motoneurons to the corresponding contralateral muscle, the simultaneous excitability changes in the antagonist motoneurons being less evident. Stimulation of Group II knee flexor afferents produces inhibition of opposite flexors, and Group III muscle afferents give a subsequent facilitation of the motoneurons of the crossed knee flexor. Electrical stimulation of the splanchnic nerves and sympathetic chain produces facilitation of monosynaptic hind leg reflexes and inhibition of polysynaptic reflexes, whereas visceral distention mainly evokes general inhibition. A long-lasting inhibition may remain several minutes after distention of the bladder.

Several earlier investigations demonstrated that acetylcholine and anticholinesterase inhibit afferent spinal reflex activity; on this basis the possibility that spinal neurons may have different chemical properties has been discussed [see e.g. (156)]. Recent observations on a transient depression of monosynaptic reflexes following close arterial injections of acetylcholine suggest that this depression is attributable to excitation of the Renshaw cells (77) which, according to Eccles *et al.* (98), are cholinergically activated from motor axon collaterals and exert an inhibitory action on the motoneurons. De Molina & Gray, however, argue that acetylcholine has spinal effects in addition to that on the Renshaw cells (238), since acetylcholine reduces dorsal horn potentials evoked by stimulation of low threshold cutaneous afferents. The latter observation is in line with the fact that acetylcholine may depress the negative cord dorsum potential evoked by stimulation of low threshold cutaneous fibres (28), since according to de Molina & Gray (237) this potential response (N_1) is identical with at least part of the negative deflection recorded from the dorsal horn.

The effect of several pharmacological substances has been tested on different spinal reflex functions (77, 107, 181). The necessity of considering secondary actions before conclusions can be drawn as to the direct action of the drugs on the spinal cord mechanisms has been thoroughly discussed and stressed by Eccles *et al.* (77). On the basis of studies on the dorsal and ventral root responses as well as on the cord potentials in the frog's isolated spinal cord, it has been concluded that the dendrites are especially sensitive to procaine (146). This effect of small doses of local anesthetics on the central neurons system is of interest *inter alia* in connection with the demonstration of their anticonvulsant action (24, 26), and the possibility that the anti-convulsant action may be caused by such an effect has also been discussed (22).

Continued studies of hypothermic effects on the spinal cord (290) demonstrate a phase of hyperresponsiveness between temperatures of 35° to 25°C., during which there is also an increase of cerebral and cerebellar evoked potentials. The hyperresponsive phase is characterized by a greater spread and loss of specificity in reflex pathways.

CEREBELLUM

Spinocerebellar afferents.—The facts that at least eight different routes may serve spinocerebellar transmission (47) and that they have both crossed and uncrossed fractions complicate the analysis of the specificity of different spinal cerebellar connections. As to the functions of the two classical "tracts" it is known that the dorsal spinal cerebellar tract is activated from skin and Group I and II muscle efferents and that there is a convergence from muscles and skin. A functional division of the dorsal tract into two fractions has been stressed (257), one for information from muscle spindles and one for Golgi tendon organ messages. Recent findings (244) confirm earlier conclusions that the ventral spinocerebellar tract conveys messages from skin, joint, and muscle although it has been stressed that the ventral tract, being monosynaptically connected to Golgi tendon organ afferents, is not activated from Group I muscle spindle afferents (258). There is also a diffuse convergence towards the ventral spinocerebellar neurons (258), and their modal aspecificity has been further stressed by Morin *et al.* (244). One of the relays through which impulses from the spinal cord are mediated to cerebellum is represented by the olive which receives mainly crossed fibres running in the ventral funiculus (243), no response being evoked by stimulation of foreleg nerves. Electrical stimulation of mixed cutaneous hind leg nerves evokes responses in the medial accessory olives of the opposite side (243). The main component of these olivary responses described by Morin *et al.* (243) is a long-lasting negative wave, especially dependent on activation of gamma-delta fibres, which is abolished by lesions of the ventral lateral funiculus ipsilateral to the olive but not by section of the dorsal cord quadrant opposite to the recording site. Grundfest *et al.* have earlier claimed that such a transection removes the short latency positive olivary potentials which they obtained in response to fore- and hind leg nerve stimulation. Morin *et al.* argue that the potentials recorded by Grundfest & Carter (141) are a result of activity in the so-called cervicothalamic pathway previously described by Morin and Thomas (240, 245). Evidence has been presented that fast cutaneous impulses from the hind leg (240) and foreleg (61) in the cat ascend not only in the dorsal funiculus [see Gardiner & Morin (125) for impulses in deep nerves] but also in the ipsilateral dorsospino-cerebellar tract in order to be relayed by collaterals to nucleus the cervicalis lateralis. According to Morin *et al.*, this nucleus (Cajal's *noyau du faisceau cerebelleux*) sends axons to the opposite side of the midbrain in order to project to the cerebral cortex. Concerning the projection of this nucleus to the cerebellum (270), results seem to be conflicting (241). The mapping of different afferent projections in the cerebellar cortex is hampered by the vari-

ability in the latency and the form of the evoked cerebellar response. The latency of the response in the paramedian lobulus and pyramis to electrical stimulation of cutaneous nerves shows spontaneous variations at the same point within the same folium, a fact which makes the evaluation of results obtained after partial spinal and transections difficult (188). Using varying stimulus strengths, frequencies, sites of stimulation, and depths of anesthesia, Morin *et al.* (242) concluded that the initial positive deflection which is occasionally present in monopolar surface records is not dependent on the type of afferent stimulation and is to be correlated with the arrival of afferent impulses at the granular layer. The subsequent more prominent and constant positive-negative deflection following the arrival of afferent impulses should signal activity in the Purkinje cell layer [cf. (6, 294)]. The conclusion that this fraction signals activity in neurons brought about exclusively by afferent inflow would make it suitable for the mapping of the afferent projection.

Cerebellar cortex.—The organization of the neurons in the cerebellar cortex [see (122, 129)] makes them of special interest for studies of the fundamental mechanisms of excitation and inhibition. Different approaches have been used. In Purpura & Grundfest's analysis (269), the differences between the cerebellar and cerebral cortices are taken into account with special reference to the action of topically and systemically administered strychnine and *d*-tubocurarine. The deductions are partially based on the concept that strychnine is a selective blockader of inhibitory synapses (268) and that it has a synergistic action with *d*-tubocurarine which also may preferentially block inhibitory synapses in different parts of the central nervous system. The hypothesis is advanced that there is relatively less inhibitory synaptic action in the cerebellar than in the cerebral cortex. The interpretation of the differential action of two "excitants", strychnine and pentylenetetrazol (Metrazol), is referred to divergent actions on the excitatory and inhibitory mechanisms. In the light of the various findings analysed in terms of the unitary factor that the cerebellar cortex possesses fewer hypopolarizing, inhibitory synaptic sites of electrogenesis than the cerebral cortex, the different synaptic organizations of the cerebellar and cerebral cortices are discussed. Purpura & Grundfest dismiss Granit & Phillip's interpretation of the "inactivation mechanism", which these authors claim is caused by excessive depolarization in their studies of the inhibition of Purkinje cell activity (135 to 137). These authors, like Albe-Fessard *et al.* (6) and Buser & Rougeul (56), have recorded unit activity in the cerebellar cortex with extracellular electrodes and ascribe the spikes obtained to single Purkinje cells responding to fastigial stimulation (causing antidromic activation of Purkinje axons) because of their short latency. The single unit responses from Purkinje cells have been further studied in the frog (228), in which three types of responses from these cells were demonstrated. The appearance of the different responses seems to result from activation by separate presynaptic systems converging on the Purkinje cell. That the nucleus fastigii represent an important relay for impulses from the vermal cortex of the anterior cerebellar

lobe is shown by anatomical (298) and physiological (283, 287, 309) investigations. Further studies on the integrative functions of this nucleus (10, 15, 247, 248) and recent investigations on the functions of brachium conjunctivum (58, 59, 279) deserve mention.

MOTOR FUNCTIONS

Ocular movements.—The state of consciousness and parameters of stimulation are important when determining the type and direction of the eye movements in response to cortical and subcortical stimulation (109, 303). That the brain stem possesses an intrinsic mechanism for co-ordinating conjugate ocular deviations is indicated by the fact that the pattern of eye movements elicited by localized electrical brain stimulation in decerebrate cats (118) resembles those found in *encéphale isolé* preparations, in which the final positions of the eyes were found to be related to the point of stimulation (164). Stimulation of the superior colliculi in decerebrate preparations results in increased responses, and it is argued that the enhancement may result from removal of inhibitory influences from the occipital cortex. It is claimed that in humans the optic tectum plays less of a role for visual reflexes to optic stimulation than in such animals as the cat in spite of a similar anatomical organization (57). Vestibular nystagmus has been studied with special reference to its dependence on extr vestibular influences (271) and the engagement of different reticular structures in the organization of nystagmus by stimulation of different loci in the brain stem (186, 187) and by recording from single reticular units during vestibular nystagmus (94, 95).

Corticospinal functions.—In a recent survey of the Sherrington school of neurophysiology, Denny-Brown remarks (83) that Sherrington took little interest in the cortical motor map as such but more in its variability "looking for some key to its functional mechanism and the way the nervous system used these excitable areas." When referring to this quotation and discussing the somatotopic organization within the sensory motor cortex of different species on the basis of stimulation experiments, one should take into account that experiments in which movements in response to cortical stimulation are observed may result in motor maps of different types depending on the stimulation technique used, as has been especially emphasized by Liddell & Phillips (200 to 202, 207). Thus, in baboons, single shock stimulation at threshold is only effective within the "arm area", eliciting contralateral flick movements of the thumb and index finger. At increasing strength, the opposable hallux, mouth, and tongue are successively involved, and the map delineated at this strength consists of overlapping "thumb-index-hallux and face areas" which cover a large part of the motor area. This is in contrast to the elaborate motor maps with narrower foci for a rich variety of peripheral movements which are obtained with repetitive stimulation (at rates such as 50 cycles per sec.), the classical motor maps [such as those of Woolsey *et al.* (310)]. The University of Wisconsin workers have expanded the scope of their data on which are based the "elaborate" motor maps in monkeys, obtained with repetitive cortical stimulation by means of an investigation

on the squirrel monkey (306). With 60 c.p.s. pulse trains, they delineated four cortical areas, within three of which a complete body representation was found: the precentral characterized by lower stimulus thresholds, the postcentral showing higher thresholds and a mirror-image pattern, the supplementary area with still higher thresholds and lacking face representation, and finally the "second" motor area coinciding with the "second" sensory area. These results are in line with those from earlier studies on, for example, the macaque (292, 310). It should also be pointed out that the muscle contractions evoked by repetitive cortical stimulation may be the result of a sum of activities in different descending systems as indicated by a series of time-spaced motor nerve responses to each stimulus in the train of shocks, the behaviour of the potential waves indicating the influence of cortically induced facilitation and inhibition (292). These considerations especially refer to results obtained on monkeys, since this species exhibits more elaborate motor maps to repetitive stimulation (as compared to single shocks) than do cats, as Livingston & Phillips (207) recently stressed; in monkeys, a direct (monosynaptic) activation of the motoneurons from corticospinal neurons has been shown (25, 27), in addition to the interneuronal mediation which is characteristic for cats (208). The conclusion concerning direct activation of motoneurons from corticospinal neurons based on electrophysiological experiments is supported by recent histological investigations on macaques (272) and chimpanzees (184).

Further information on the corticospinal control of movements has been obtained by intracellular recording of the activity in spinal motoneurons and interneurons following stimulation of corticospinal neurons at the pyramidal level in cats, demonstrating both excitatory and inhibitory effects (246). The necessity of discarding the conception of the "pyramidal tract" as a single entity and, consequently, also the "pyramidal syndrome" when considering neuronal mechanisms for motor control and their disturbance has been stressed by Bucy (50, 51) on the basis of studies on the relation between motor disturbances and localization of lesions.

During the last year, further physiological and anatomical data have been presented concerning centrifugal influences on various sensory transmission systems. It has been found in stimulation experiments on cats that the pyramidal tract may mediate descending influences on the primary afferent relay in the spinal cord (206) and in degeneration experiments on the same animal that corticifugal fibres descend in the pyramidal tract to the dorsal column nuclei (304).

RETICULAR FORMATION

Two excellent and comprehensive reviews, one by Rossi & Zanchetti (276) and one by Brodal (44), are devoted to anatomical and physiological aspects of the reticular formation, i.e., of the appropriate structures of the medulla, pons, and mesencephalon. Their broad outlines are based on the vast experimental work from different laboratories for which the early observations and considerations by Magoun and co-workers represent a

triggerlike impetus in reticular formation physiology. Rossi and Zanchetti also discuss the integration between the reticular formation and the diencephalic structures towards which interest was directed by Morison & Dempsey's demonstration of the cortical recruiting response to intralaminar thalamic stimulation (80, 81). In their concluding remarks, when pointing out that it is unlikely that the reticular formation functions indiscriminately as an "energizer" only, they quote Adrian (2), who questioned whether the ascending reticular system has nothing more to do than

just coming in to wake us up in the morning and to send us to sleep at night, to do nonspecific activation, or whether it might have something to do with the direction attention, with the actual work of the conscious brain.

Evarts & Magoun (116), after confirming the observation by Jasper *et al.* (169) that reduction of the recruiting response followed habituation, reported that, in contrast to earlier assumptions, the presence of "desynchronized" cortical activity indicative of an alert waking state is not incompatible with the elicitation of recruiting responses by thalamic stimulation. They openmindedly remark that

the effect of brain stem stimulation has customarily been given blanket equation with awakening but depending on the state of the animal and the parameters and location of stimulation, such stimulation would appear to be capable of leading to a wide spectrum of behavioural changes that range from awakening to evocation of attention, to startle, or to the arousal of generalized excitement.

Both physiological (12, 128, 205, 288) and anatomical (44, 275) findings show that there is a fractional organization of the reticular formation (154) and that both ascending and descending reticular influences are less diffuse than was previously assumed. Austin & Jasper (12), in their work on the facilitatory and inhibitory effects of upper brainstem stimulation on cats in chloralose pentobarbital sodium (Nembutal) anesthesia, remark that the use of different narcotics and anesthetic depths may be the cause of discrepancies in the results concerning the effects of brainstem stimulation. In this context, it should be mentioned that on the basis of experiments on different types of preparation it is concluded that chemical (anesthetic) or surgical (decerebrate) removal of suprabulbar structures is required for the demonstration of bulbar reticular inhibition of spinal cord reflex activity (225). Torvik & Brodal (300) and Brodal & Gogstad (45) have investigated the connections of these reticular areas histologically by studying the distribution of the degenerations following localized lesions.

It has further been emphasized that the nonspecific thalamic nuclei should not be regarded as an integral part of the reticular ascending system [see Rossi & Zanchetti (276)] but that they merely represent one structure which is influenced from one part of the reticular formation (medulla and pons), the hypothalamus and basal ganglia being other such pools which receive influences probably from other parts (midbrain and rostral midbrain respectively). Investigations in rabbits of the thalamic source of the cortical recruiting response (176) confirm earlier results on cats and monkeys, dem-

onstrating the importance of the thalamic midline nuclei for the recruiting response. The role played by the basal ganglia in this type of cortical activation is considered in investigations demonstrating, on the one hand, that responses in the striatum and pallidum appear as a consequence of thalamic stimulation (286) and, on the other hand, that uni- and bilateral destruction of the globus pallidus does not prevent the appearance of cortical recruitment caused by thalamic stimulation in pentobarbitalized cats (295). Behavioural and electrocortical changes have been further studied after stimulation of reticular formation, diencephalon, subthalamus, dorsal hypothalamus, and ventrolateral thalamus (265). The role of the centre median (CM) as a relay for messages from cutaneous and muscular afferents to the nonspecific cortical projection areas has been discussed on the basis of experiments in which the characteristics of the responses of these areas were compared with those of the CM responses (5). The hypothesis is advanced (276) of a differentiation into a "phasic" fraction characterized by short-latency rapidly conducted volleys in long reticulospinal fibres with highly organized patterns in space and time, and a tonic fraction of activity mediated in slowly conducted volleys in polysynaptic pathways which, in a less specific manner, modulate the level of the excitability in certain spinal cord neurons. Interesting data are being collected concerning the influence of the reticular formation on the spinal levels by intracellular recording of excitatory and inhibitory actions on spinal interneurons and motoneurons elicited by reticular stimulation (291). In experiments on nonanesthetized cats with implanted electrodes, the inhibitory influence from the reticular formation on photically evoked responses at different levels of the optic pathways has been studied (152) and, on the basis of experiments in which the electrodes were in the dorsal cochlear nucleus (153), it is concluded that habituation involves inhibition at the specific afferent pathway for which the integrity of the brainstem reticular system is required.

Experiments on cats and rabbits show that gamma motoneurons innervating the muscle spindles are also influenced from hypothalamic thermosensitive structures, and the brain stem reticular formation is regarded as the relay system (112).

Homologous with the spinal monosynaptic reflex, a monosynaptic masseter reflex has been demonstrated, the characteristics of which have been described earlier (159, 221, 296). Hugelin & Bonvallet (160 to 163), in a series of interesting investigations, used this reflex to test motoneuron excitability during suprabulbar reticular stimulation in *encéphale isolé* preparations. In such preparations, reticular stimulation in the tegmentum elicits the well known facilitation of the monosynaptic reflex which, however, is counteracted by inhibition, the cortical origin of which is demonstrated by decortication or cortical cooling. This cortical inhibitory control seems to be independent of the reticular formation inhibition and seems to coincide with the cortical EEG arousal. The hypothesis is thus advanced postulating the existence of a control mechanism consisting of a reticulo-corticoreticular counteraction circuit including the ascending activating system, an interneuronic cortical loop, and a corticofugal inhibitory path

which restrains the reticular activity. On the basis of the effect of localized lesions, it is concluded that the subcortical course of the corticofugal inhibitory effect is mediated via the internal capsule and the lateral hypothalamic area in order to reach the posterior diencephalic tegmentum.

Concerning the humoral activation of reticulospinal systems, earlier investigations have demonstrated that epinephrine stimulates reticular neurons with descending influence on the excitability of motoneurons (79, 281) and produces EEG arousal as a result of influences on mesencephalic structures (32, 277). On the basis of recent findings, it is assumed that methamphetamine and cocaine effects are attributable to their influence on the adrenergic component of the reticular activating system (278). There is an increasing literature on the central effect of chlorpromazine, and it is generally ascribed to the action of the reticular activating system (124), which action has been related to antiepinephrine properties (34). On the basis of investigations into the effect of different drugs, including amphetamine, lysergic acid diethylamide, atropine, and physostigmine on the cortical activity and behaviour in conscious animals, as well as in acute preparations with transections at different levels, the possibility that there may be several different receptors in the reticular formation and diencephalic structures has been discussed (33, 35).

INTRINSIC CORTICAL MECHANISMS

The specificity of regional corticographic patterns has been studied with implanted electrodes in monkeys (182). The results support the findings in man that the central fissure forms an approximate boundary for slow activity recorded from the parietal and occipital cortex.

Investigations of *encéphale isolé* preparations demonstrate the role of the corpus callosum and the anterior commissure for the synchronization of cortical activity (39, 40). On the basis of training experiments in cats, it is concluded that the corpus callosum is essential for the contralateral transfer of somesthetic discrimination from one forepaw to the other in this animal (289), there being a species difference in this respect between cats and dogs on the one hand and humans on the other.

Burns (54) has recently reviewed his interesting investigations on isolated parts of the cerebral cortex with intact pial blood supply, which he and his collaborators have successfully used for the analysis of the transmission of excitation within the mammalian cerebral cortex. It should be noted that there is relatively little alteration in the electrophysiological properties of such isolated cortical slabs two to eight weeks after isolation, judging by different types of responses to electrical cortical stimulation, and that such isolated parts exhibit a long-lasting electrically evoked epileptiform after-discharge (132). A technique for maintaining wholly isolated mammalian tissues for electrophysiological investigation has been described, and intracellularly recorded membrane potentials ranging from 1 to 91 mv. are reported (199). No spontaneous activity or response to electrical stimulation was obtained, but injury discharges were occasionally found.

According to Clare & Bishop (64, 65), the cortical recruiting described by

Morison and Dempsey is of dendritic origin. They have shown that this response is graded and can be summated to a prolonged steady state. Goldring & O'Leary (131), who have studied the cortical direct current shift following stimulation of the midline thalamus in rabbits with frequencies eliciting recruiting activity, found a negative shift of 1.5 mv. persisting for several seconds after cessation of stimulation. This is interpreted as a summated steady state of activity in the dendrites. Depending on the stimulation parameters, both negative and positive direct current shifts were obtained which were associated with excitability changes. Studies on the steady potential difference between the cortical surface and a reference point, its changes caused by afferent stimulation (185) and different pathological states (53, 84, 147, 234 to 236, 302), as well as its relation to cortical convulsive activity (131, 147, 302), are of interest in connection with the physiological significance of maintained cortical polarization states (126). Observations on the ontogenetic development of the steady potential (52) indicate a relationship between the degree of cortical polarization and its functional maturity. Clare & Bishop (66) hold the view that strychnine has two effects on the cortical activity: (a) a depressing effect by lowering the power of the presynaptic terminals to stimulate the postsynaptic locus and by lowering the dendritic response, whether activated synaptically or via the cell body; and (b) an excitability-increasing effect on the dendrites themselves. They also conclude that the synapses of the nonmyelinated fibres with the apical dendrites, which according to them mediate the long-latency recruiting response, are especially susceptible to strychnine. The impressive series of investigations on central synaptic transmission by Grundfest and Purpura, which leads to a divergent opinion concerning the properties of cortical dendrites, was summarized by Mountcastle (250). Since then Grundfest (140) has published a review on synaptic transmission, the main conclusion of which is that

the major phenomena which distinguish central nervous activity from that of nerve or muscle can be accounted for by two properties of synaptic electrogenesis: (a) that it is chemically excitable, not electrically; and (b) that either depolarizing or hyperpolarizing electrogenesis can occur, depending on the subclass of synaptic membrane.

In regard to the effect of strychnine, Purpura & Grundfest (268) hold the view that it selectively blocks inhibiting synapses, as does *d*-tubocurarine when the excitatory synapses are protected by heparin. This would be in contrast to γ -aminobutyric acid which has a depressant action on the superficial cortical layers (166), and which according to Purpura, Girado & Grundfest (266, 267) blocks depolarizing excitatory synaptic electrogenesis in the mammalian brain, in line with the effect on the mechanosensitive receptor (20, 108, 111, 121).

LIMBIC SYSTEM

Clinical, anatomical, and physiological investigations have led to interest in the role of the limbic system (260) for memory, learning, and affective behaviour. In line with earlier observations, recent investigations with chemi-

cal and electrical stimulation demonstrate the involvement of the hippocampus in pleasure and grooming reactions in the cat (222, 223). Functional relationships with the hypothalamus (130) are demonstrated by recording hypothalamic seizure activity in response to stimulation of the lateral amygdaloid nucleus, hippocampus, and septum (264) as well as by the study of behavioural changes attributable to destruction of rhinencephalic-hypothalamic connections (31). Comparative studies on behaviour changes evoked by electrical stimulation of the anterior and cingulate gyrus on one hand and the hippocampus on the other have also been reported (9), and the role played by hippocampal injury for temporal lobe seizures has been tested in cats (139). In the study of the mechanism underlying emotional behaviour, great interest has been shown in the interconnections between hippocampus and the entorhinal area of the hippocampal gyrus, as well as to their interrelations with midbrain structures via the fornix and stria medullaris. Recent electrophysiological experiments (1) on the reciprocity between the entorhinal area, hippocampus, and fornix in the marsupial phalanger confirm earlier conclusions concerning pathways mediating messages from thalamic nuclei via the fornix to the hippocampus and the entorhinal area, and also concerning activation of the midbrain tegmentum from the entorhinal area via the stria medullaris. Transmission in the opposite direction, from the entorhinal area through the hippocampus to the fornix, seems to be doubtful since entorhinal stimulation gives inconsistent responses in the fornix. Shealy & Peele (280) did not obtain sham rage in cats after destruction of the amygdala, although this had been observed in earlier investigations (13). They report, however, that the destructions were never complete. In conformity with earlier investigations, they found that stimulation of the amygdala in nonanesthetized cats evoked somatic (head, facial jaw, and tongue movements) and behavioural reactions (alertness reaction, fear, or rage) and widespread autonomic responses. It is known that the threshold for seizure activity in rhinencephalic structures is low and that carbon dioxide influences seizure activity. The spontaneous and evoked activity of especially the ventral hippocampus has been found to be depressed by carbon dioxide, the fornix-hippocampal system being more susceptible than the thalamic cortical relay system (96, 97). Comparative studies of the effect of reserpine on rhinencephalic activity and on the thalamocortical recruiting response have also been made (282).

LITERATURE CITED

1. Adey, W. R., Sunderland, S., and Dunlop, C. W., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 309-24 (1957)
2. Adrian, E. D., Bremer, F., and Jasper, H. H., *Brain Mechanisms and Consciousness* (Basil Blackwell & Mott, Ltd., Oxford, Engl., 456 pp., 1954)
3. Akimoto, H., and Creutzfeldt, O., *Klin. Wochschr.*, **35**, 199 (1957)
4. Akimoto, H., and Creutzfeldt, O., *Arch. Psychiat. Nervenkrankh.*, **196**, 494-519 (1958)
5. Albe-Fessard, D., and Rouguet, A., *Electroencephalog. and Clin. Neurophysiol.*, **10**, 131-52 (1958)

6. Albe-Fessard, D., and Szabo, T., *Compt. rend. soc. biol.*, **149**, 1090-93 (1955)
7. Amassian, V. E., and Devito, V., *J. Neurophysiol.*, **17**, 575-603 (1954)
8. Andersson, B., and Jewell, P. A., *J. Physiol. (London)*, **139**, 191-97 (1957)
9. Andy, O. J., and Chinn, R. McC., *Neurology*, **7**, 56-68 (1957)
10. Arduini, A., and Pompeiano, O., *Rev. arch. ital. biol.*, **95**, 56-70 (1957)
11. Arief, A. J., Pyzik, S. W., and Tigay, E. I., *Arch. Neurol. Psychiat.*, **77**, 156-58 (1957)
12. Austin, G., and Jasper, H., *Neurology*, **7**, 615-24 (1957)
13. Bard, P., and Mountcastle, V. B., *Research Publ., Assoc. Research Nervous Mental Disease*, **27**, 362-404 (1947)
14. Barlow, H. B., Fitzhugh, R., and Kuffler, S. W., *J. Physiol. (London)*, **137**, 338-54 (1957)
15. Batini, C., Moruzzi, G., and Pompeiano, O., *Rev. arch. ital. biol.*, **95**, 71-95 (1957)
16. von Baumgarten, R., and Mollica, A., *Arch. ges. Physiol.*, **259**, 79-96 (1954)
17. von Baumgarten, R., and Schaefer, K. P., *Arch. ges. Physiol.*, **265**, 264-75 (1957)
18. von Baumgarten, R., and Schaefer, K. P., *Arch. ges. Physiol.*, **266**, 7-8 (1957)
19. Baumgartner, G., *Arch. ges. Physiol.*, **261**, 456-69 (1955)
20. Bazemore, A., Elliott, K. A. C., and Florey, E., *Nature*, **178**, 1052-53 (1956)
21. Benjamin, R. M., and Welker, W. I., *J. Neurophysiol.*, **20**, 286-99 (1957)
22. Bernhard, C. G., *Exptl. Cell Research*, Suppl. 5, 201-20 (1958)
23. Bernhard, C. G., *Acta Physiol. Scand.*, **29**, 1-29 (1952)
24. Bernhard, C. G., and Bohm, E., *Experientia*, **10**, 474-75 (1954)
25. Bernhard, C. G., and Bohm, E., *Arch. Neurol. Psychiat.*, **72**, 473-502 (1954)
26. Bernhard, C. G., Bohm, E., Kirstein, L., and Wiesel, T., *Arch. intern. pharmacodymie*, **108**, 408-19 (1956)
27. Bernhard, C. G., Bohm, E., and Petersén, I., *Acta Physiol. Scand.*, **29**, 79-105 (1953)
28. Bernhard, C. G., and Skoglund, C. R., *Acta Physiol. Scand.*, **29**, Suppl. 106, 435-54 (1953)
29. Beswick, F. B., and Evanson, J. M., *J. Physiol. (London)*, **135**, 400-11 (1957)
30. Bishop, G. H., *Physiol. Revs.*, **26**, 77-102 (1947)
31. Bond, D. D., Randt, C. T., Bidder, G. T., and Rowland, V., *Arch. Neurol. Psychiat.*, **78**, 143-62 (1957)
32. Bonvallet, M., Dell, P., and Hiebel, G., *Electroencephalog. and Clin. Neurophysiol.*, **6**, 119-44 (1954)
33. Bradley, P. B., and Elkes, J., *Brain*, **80**, 77-117 (1957)
34. Bradley, P. B., and Hance, A. J., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 191-215 (1957)
35. Bradley, P. B., and Key, B. J., *Electroencephalog. and Clin. Neurophysiol.*, **10**, 97-110 (1958)
36. Bremer, F., *Arch. intern. physiol.*, **53**, 53-103 (1943)
37. Bremer, F., *Les aires auditives de l'écorce cérébrale*, 151-71 (Norbert Maloine, Paris, France, 1952)
38. Bremer, F., and Bonnet, V., *Electroencephalog. and Clin. Neurophysiol.*, **1**, 447-49 (1949)
39. Bremer, F., and Stoupel, N., *Acta Physiol. et Pharmacol. Neerl.*, **6**, 487-96 (1957)
40. Bremer, F., and Stoupel, N., *J. physiol. et pathol. gén.*, **49**, 66-67 (1957)
41. Brindley, G. S., *Symposium on Visual Problems of Colour* (Nat. Phys. Lab., Teddington, Middlesex, Engl. 1957)

42. Brindley, G. S., *Ann. Rev. Physiol.*, **20**, 559-82 (1958)
43. Brindley, G. S., *J. Physiol. (London)*, **140**, 247-61 (1958)
44. Brodal, A., in the William Ramsey Henderson Trust lectures (Oliver and Boyd, Ltd., London, Engl., 74 pp., 1957)
45. Brodal, A., and Gogstad, A. C., *Acta Anat.*, **30**, 133-51 (1957)
46. Brodal, A., and Pompeiano, O., *J. Anat.*, **91**, 438-54 (1957)
47. Brodal, A., and Rexed, B., *J. Comp. Neurol.*, **98**, 179-211 (1953)
48. Brodal, A., and Torvik, A., *Arch. Psychiat. Nervenkrankh.*, **195**, 550-67 (1957)
49. Brown, K. T., and Wiesel, T. N., *Federation Proc.*, **16**, 68 (1957)
50. Bucy, P. C., *Acta Neurol. Latinoam.*, **3**, 157-71 (1957)
51. Bucy, P. C., *Brain*, **80**, 376-92 (1957)
52. Bures, J., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 121-30 (1957)
53. Bures, J., and Burešová, O., *Arch. ges. Physiol.*, **264**, 325-34 (1957)
54. Burns, B. D., *The Mammalian Cerebral Cortex* (Edward Arnold & Co., London, Engl., 1958)
55. Buser, P., *J. physiol. et pathol. gén.*, **49**, 589-656 (1957)
56. Buser, P., and Rougeul, A., *J. Physiol. (London)*, **46**, 287-91 (1954)
57. Bürgi, S., *Z. Nervenheilk.*, **176**, 701-29 (1957)
58. Carpenter, M. B., *Neurology*, **7**, 427-37 (1957)
59. Carpenter, M. B., and Stevens, G. H., *J. Comp. Neurol.*, **107**, 109-52 (1957)
60. Case, J. F., Edwards, C., Gesteland, R., and Ottoson, D., *Biol. Bull.*, **2**, 360 (1957)
61. Catalano, J. V., and Lamarche, G., *Am. J. Physiol.*, **189**, 141-44 (1957)
62. Catton, W. T., *J. Physiol. (London)*, **141**, 305-22 (1958)
63. Chang, H. T., *Research Pubs. Assoc. Nervous Mental Disease*, **30**, 430-53 (1952)
64. Clare, M. H., and Bishop, G. H., *Electroencephalog. and Clin. Neurophysiol.*, **8**, 85-98 (1955)
65. Clare, M. H., and Bishop, G. H., *Electroencephalog. and Clin. Neurophysiol.*, **8**, 583-602 (1956)
66. Clare, M. H., and Bishop, G. H., *J. Neurophysiol.*, **20**, 255-74 (1957)
67. Cohen, M. J., Landgren, S., Ström, L., and Zotterman, Y., *Acta Physiol. Scand.*, **40**, 3-50 (1957)
68. Coleman, P. D., *An Electrophysiological Study of the Interaction Between Responses to Successive Clicks in the Inferior Colliculus of the Cat* (Unpublished Ph.D. dissertation, Univ. of Rochester, Rochester, N. Y., 1953); cited in Rosenzweig, M. R., and Sutton, D., *J. Neurophysiol.*, **21**, 17-23 (1958)
69. Cooper, S., and Daniel, P. M., *Quart. J. Exptl. Physiol.*, **42**, 222-31 (1957)
70. Cooper, S., Daniel, P. M., and Whitteridge, D., *J. Physiol. (London)*, **120**, 471-90 (1953)
71. Cooper, S., Daniel, P. M., and Whitteridge, D., *Brain*, **78**, 564-83 (1955)
72. Cooper, S., Daniel, P. M., and Whitteridge, D., *J. Physiol. (London)*, **133**, 1-3 (1956)
73. Creutzfeldt, O., *Abstr. Congr. intern. Physiol. Meeting XX* (Brussels, 1956)
74. Creutzfeldt, O., and Akimoto, H., *Arch. Psychiat. Nervenkrankh.*, **196**, 520-38 (1958)
75. Creutzfeldt, O., and Baumgartner, G., *Electroencephalog. and Clin. Neurophysiol.*, **7**, 664 (1955)
76. Creutzfeldt, O., and Grüsser, O.-J., *Abstr. Cong. intern. Sci. neurol., Meeting I* (Brussels, 1957)

77. Curtis, D. R., Eccles, J. C., and Eccles, R. M., *J. Physiol. (London)*, **136**, 420-34 (1957)
78. Deane, H. W., Enroth-Cugell, C., Gongaware, M. S., Neyland, M., and Forbes, A., *J. Neurophysiol.*, **21**, 45-61 (1958)
79. Dell, P., Bonvallet, M., and Hugelin, A., *Electroencephalog. and Clin. Neurophysiol.*, **6**, 599-618 (1954)
80. Dempsey, E. W., and Morison, R. S., *Am. J. Physiol.*, **135**, 301-08 (1942)
81. Dempsey, E. W., and Morison, R. S., *Am. J. Physiol.*, **138**, 283-96 (1943)
82. Denny-Brown, D., *Proc. Roy. Soc. (London). B.*, **104**, 252-301 (1929)
83. Denny-Brown, D., *J. Neurophysiol.*, **20**, 543-48 (1957)
84. Denny-Brown, D., and Meyer, J. S., *Neurology*, **7**, 567-79 (1957)
85. Diamond, J., Gray, J. A. B., and Inman, D. R., *J. Physiol. (London)*, **138**, 51-32 (1957)
86. Diamond, J., Gray, J. A. B., and Inman, D. R., *J. Physiol. (London)*, **141**, 117-31 (1958)
87. Dodt, E., and Walther, J. B., *Arch. ges. Physiol.*, **265**, 355-64 (1957)
88. Dodt, E., and Walther, J. B., *Arch. ges. Physiol.*, **266**, 167-74 (1958)
89. Dodt, E., and Walther, J. B., *Arch. ges. Physiol.*, **266**, 175-86 (1958)
90. Douglas, W. W., and Ritchie, J. M., *J. Physiol. (London)*, **135**, 19-30 (1957)
91. Douglas, W. W., and Ritchie, J. M., *J. Physiol. (London)*, **138**, 31-43 (1957)
92. Douglas, W. W., and Ritchie, J. M., *J. Physiol. (London)*, **139**, 385-99 (1957)
93. Douglas, W. W., and Ritchie, J. M., *J. Physiol. (London)*, **139**, 400-7 (1957)
94. Duensing, F., and Schaefer, K. P., *Arch. Psychiat. Nervenkrankh.*, **196**, 265-90 (1957)
95. Duensing, F., and Schaefer, K. P., *Arch. Psychiat. Nervenkrankh.*, **196**, 402-20 (1957)
96. Dunlop, C. W., *Am. J. Physiol.*, **190**, 172-76 (1957)
97. Dunlop, C. W., *Am. J. Physiol.*, **191**, 200-2 (1957)
98. Eccles, J. C., Eccles, R. M., and Fatt, P., *J. Physiol. (London)*, **131**, 154-69 (1956)
99. Eccles, J. C., Eccles, R. M., and Lundberg, A., *J. Physiol. (London)*, **136**, 527-46 (1957)
100. Eccles, J. C., Eccles, R. M., and Lundberg, A., *J. Physiol. (London)*, **137**, 22-50 (1957)
101. Eccles, J. C., Eccles, R. M., and Lundberg, A., *J. Physiol. (London)*, **138**, 227-52 (1957)
102. Eccles, J. C., Eccles, R. M., and Lundberg, A., *Nature*, **179**, 866-68 (1957)
103. Eccles, J. C., Fatt, P., and Koketsu, K., *J. Physiol. (London)*, **126**, 524-62 (1954)
104. Eccles, J. C., Fatt, P., and Landgren, S., *Australian J. Sci.*, **16**, 130 (1954)
105. Eccles, J. C., Fatt, P., and Landgren, S., *J. Neurophysiol.*, **19**, 75-98 (1956)
106. Eccles, R. M., and Lundberg, A., *Nature*, **179**, 1305-6 (1957)
107. Edisen, C. B., *Arch. Neurol. Psychiat.*, **79**, 323-27 (1958)
108. Edwards, C., and Kuffler, S. W., *Federation Proc.*, **16**, 145 (1957)
109. Eliasson, S. G., Hyde, J. E., and Bach-y-Rita, P., *Am. J. Physiol.*, **191**, 203-8 (1957)
110. Eliot, C. R., Kaji, A., Seeman, P., Ubell, E., Kuffler, S. W., and Burgen, A. S. V., *Biol. Bull.*, **113**, 344 (1957)
111. Elliott, K. A. C., and Florey, E., *J. Neurochem.*, **1**, 181-91 (1956)
112. von Euler, C., and Söderberg, U., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 391-408 (1957)

113. Evans, M. H., and McPherson, A., *J. Physiol. (London)*, **140**, 201-12 (1958)
114. Evarts, E. V., and Hughes, J. R., *Am. J. Physiol.*, **188**, 238-44 (1957)
115. Evarts, E. V., and Hughes, J. R., *Am. J. Physiol.*, **188**, 245-48 (1957)
116. Evarts, E. V., and Magoun, H. W., *Science*, **125**, 1147-48 (1957)
117. Eyzaguirre, C., *J. Neurophysiol.*, **20**, 523-42 (1957)
118. Faulkner, R. F., and Hyde, J. E., *J. Neurophysiol.*, **21**, 171-82 (1958)
119. Feldberg, W., Malcolm, J. L., and Smith, I. D., *J. Physiol. (London)*, **138**, 178-201 (1957)
120. Fernand, V. S. V., and Young, J. Z., *Proc. Roy. Soc. (London). B*, **139**, 38-58 (1951)
121. Florey, E., *Arch. intern. physiol.*, **62**, 33-53 (1954)
122. Fox, C. A., and Barnard, J. W., *J. Anat.*, **91**, 299-313 (1957)
123. Frank, K., and Fuortes, M. G., *J. Physiol. (London)*, **131**, 424-35 (1956)
124. Gangloff, H., and Monnier, M., *Helv. Physiol. et Pharmacol. Acta*, **15**, 83-104 (1957)
125. Gardner, E., and Morin, F., *Am. J. Physiol.*, **189**, 152-58 (1957)
126. Gerard, R. W., and Libet, B., *Am. J. Psychiat.*, **96**, 1127-52 (1940)
127. Gernandt, B. E., Katsuki, Y., and Livingston, R. B., *J. Neurophysiol.*, **20**, 453-69 (1957)
128. Gernandt, B. E., and Thulin, C.-A., *J. Neurophysiol.*, **18**, 113-29 (1955)
129. Glees, P., Pearson, C., and Smith, A. G., *Quart. J. Exptl. Physiol.*, **43**, 52-60 (1958)
130. Gloor, P., *Electroencephalog. and Clin. Neurophysiol.*, **7**, 223-42 (1955)
131. Goldring, S., and O'Leary, J. L., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 577-84 (1957)
132. Grafstein, B., and Sastry, P. B., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 723-25 (1957)
133. Granit, R., Henatsch, H. D., and Steg, G., *Acta Physiol. Scand.*, **37**, 114-26 (1956)
134. Granit, R., Pascoe, J. E., and Steg, G., *J. Physiol. (London)*, **138**, 381-400 (1957)
135. Granit, R., and Phillips, C. G., *J. Physiol. (London)*, **132**, 58-59 (1956)
136. Granit, R., and Phillips, C. G., *J. Physiol. (London)*, **133**, 520-47 (1956)
137. Granit, R., and Phillips, C. G., *J. Physiol. (London)*, **135**, 73-92 (1957)
138. Granit, R., Phillips, C. G., Skoglund, S., and Steg, G., *J. Neurophysiol.*, **20**, 470-81 (1957)
139. Green, J. D., Clemente, C. D., and de Groot, J., *Arch. Neurol. Psychiat.*, **78**, 259-63 (1957)
140. Grundfest, H., *Physiol. Revs.*, **37**, 337-61 (1957)
141. Grundfest, H., and Carter, W. B., *J. Neurophysiol.*, **17**, 72-91 (1954)
142. Grüsser, O.-J., *Naturwissenschaften*, **19**, 522-23 (1957)
143. Grüsser, O.-J., and Kapp, H., *Arch. ges. Physiol.*, **266**, 11-129 (1958)
144. Grüsser, O.-J., and Rabelo, C., *Arch. ges. Physiol.*, **265**, 501-25 (1958)
145. Hammond, P. H., Merton, P. A., and Sutton, G. G., *Brit. Med. Bull.*, **12**, 214-18 (1956)
146. Harmel, M., and Malcolm, J. L., *J. Physiol. (London)*, **140**, 213-19 (1958)
147. van Harreveld, A., and Ochs, S., *Am. J. Physiol.*, **189**, 159-66 (1957)
148. Hartline, H. K., and Ratliff, F., *J. Gen. Physiol.*, **40**, 357-76 (1957)
149. Hartline, H. K., Wagner, H. G., and Ratliff, F., *J. Gen. Physiol.*, **39**, 651-73 (1956)
150. Heck, J., *Acta Physiol. Scand.*, **39**, 158-66 (1957)

151. Heck, J., and Rendahl, I., *Acta Physiol. Scand.*, **39**, 167-75 (1957)
152. Hernández-Peón, R., Guzmán-Flores, C., Alcaez, M., and Fernández-Guardiola, A., *Acta neurol. Latinoam.*, **3**, 1-8 (1957)
153. Hernández-Peón, R., and Scherrer, H., *Acta neurol. Latinoam.*, **3**, 144-56 (1957)
154. Hess, V. R., *Arch. Psychiat. Nervenkrankh.*, **196**, 329-36 (1957)
155. Holmgren, B., and Merton, P. A., *J. Physiol. (London)*, **123**, 47 P (1954)
156. Holmstedt, B., and Skoglund, C. R., *Acta Physiol. Scand.*, **29**, Suppl. 106, 410-34 (1953)
157. Hubbard, S. J., *J. Physiol. (London)*, **137**, 40-41 (1957)
158. Hubbard, S. J., *J. Physiol. (London)*, **141**, 198-218 (1958)
159. Hugelin, A., and Bonvallet, M., *Compt. rend. soc. biol.*, **150**, 2067-71 (1956)
160. Hugelin, A., and Bonvallet, M., *J. physiol. pathol. gén.*, **49**, 212-14 (1957)
161. Hugelin, A., and Bonvallet, M., *J. physiol. pathol. gén.*, **49**, 1171-1200 (1957)
162. Hugelin, A., and Bonvallet, M., *J. physiol. pathol. gén.*, **49**, 1201-23 (1957)
163. Hugelin, A., and Bonvallet, M., *J. physiol. pathol. gén.*, **49**, 1225-34 (1957)
164. Hyde, J. E., and Eliasson, S. G., *J. Comp. Neurol.*, **108**, 139-72 (1957)
165. Häggqvist, G., *Z. mikroskop.-anat. Forsch. (Abt. 2 Jahrb. Morphol. Mikroskop. Anat.)*, **44**, 169-86 (1938)
166. Iwama, K., and Jasper, H. H., *J. Physiol. (London)*, **138**, 365-80 (1957)
167. Jack, J. J. B., *Proc. Univ. Otago Med. School.*, **35**, 33-35 (1957)
168. Jasper, H., and Ajmone-Marsan, C., *Research Publ. Assoc. Research Nervous Mental Disease*, **30**, 493-512 (1952)
169. Jasper, H., Naquet, R., and King, E. E., *Electroencephalog. and Clin. Neurophysiol.*, **7**, 99-114 (1955)
170. Johnson, D. A., and Bickford, R. G., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 251-62 (1957)
171. Jung, R., and Baumgartner, G., *Arch. ges. Physiol.*, **261**, 434-56 (1955)
172. Jung, R., Creutzfeldt, O., and Baumgartner, G., *Colloq. Microphysiol. des Systèmes excitables*, 411-34 (C.N.R.S., Paris, France, 1957)
173. Jung, R., Creutzfeldt, O., and Grüsser, O.-J., *Deut. med. Wochschr.*, **82**, 1050-59 (1957)
174. Keidel, W. D., *Arch. ges. Physiol.*, **264**, 17-30 (1957)
175. Keidel, W. D., *Arch. ges. Physiol.*, **264**, 31-43 (1957)
176. Kerr, F. W. L., and O'Leary, J., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 461-76 (1957)
177. Koketsu, K., and Nishi, S., *J. Physiol. (London)*, **137**, 193-209 (1957)
178. Koketsu, K., and Nishi, S., *J. Physiol. (London)*, **139**, 15-26 (1957)
179. Kolmodin, G. M., *Acta Physiol. Scand.*, **40**, 5-87 (1957)
180. Kolmodin, G. M., and Skoglund, C. R., *Experientia*, **10**, 505-6 (1954)
181. Krivoy, W. A., *Proc. Soc. Exptl. Biol. Med.*, **96**, 18-20 (1957)
182. Kruger, L., and Henry, C., *Neurology*, **7**, 490-95 (1957)
183. Kuffler, S. W., *J. Neurophysiol.*, **16**, 37-68 (1953)
184. Kuypers, H. G. J. M. (Personal communication, 1957)
185. Köhler, W., and O'Connell, D. N., *J. Cellular Comp. Physiol.*, **49**, 1-43 (1957)
186. Lachmann, J., Bergmann, F., and Monnier, M., *Helv. Physiol. et Pharmacol. Acta*, **15**, C5-C6 (1957)
187. Lachmann, J., Bergmann, F., and Monnier, M., *J. physiol. et pathol. gén.*, **49**, 248 (1957)
188. Lamarche, G., and Morin, F., *J. Neurophysiol.*, **20**, 274-85 (1957)

189. Landgren, S., *Acta Physiol. Scand.*, **40**, 202-9 (1957)
190. Landgren, S., *Acta Physiol. Scand.*, **40**, 210-21 (1957)
191. Laporte, Y., and Lloyd, D. P. C., *Am. J. Physiol.*, **169**, 609-12 (1952)
192. Legoux, J. P., *Science*, **123**, 331-35 (1956)
193. Legoux, J. P., *J. physiol. (Paris)*, **49**, 262-65 (1957)
194. Lennox, M. A., *J. Neurophysiol.*, **19**, 271-79 (1956)
195. Lennox, M. A., *J. Neurophysiol.*, **21**, 62-69 (1958)
196. Lennox, M. A., *J. Neurophysiol.*, **21**, 70-84 (1958)
197. Lennox, M. A., and Madsen, A., *J. Neurophysiol.*, **18**, 412-24 (1955)
198. Li, C. L., *J. Physiol. (London)*, **131**, 115-24 (1956)
199. Li, C. L., and Mellwain, H., *J. Physiol. (London)*, **139**, 178-90 (1957)
200. Liddell, E. G. T., and Phillips, C. G., *Brain*, **73**, 125-40 (1950)
201. Liddell, E. G. T., and Phillips, C. G., *J. Physiol. (London)*, **112**, 392-99 (1957)
202. Liddell, E. G. T., and Phillips, C. G., *Brain*, **75**, 510-25 (1952)
203. Liljestrand, G., and Magnus, R., *Arch. ges. Physiol.*, **175**, 168-208 (1919)
204. Lindblom, U. F., *Arch. Physiol. Scand.*, **42**, Suppl. 145, 97-98 (1957)
205. Lindblom, U. F., and Ottoson, J. O., *Acta Physiol. Scand.*, **35**, 203-14 (1956)
206. Lindblom, U. F., and Ottoson, J. O., *Acta Physiol. Scand.*, **38**, 309-18 (1957)
207. Livingston, A., and Phillips, C. G., *Quart. J. Exptl. Physiol.*, **42**, 190-205 (1957)
208. Lloyd, D. P. C., *J. Neurophysiol.*, **4**, 525-46 (1941)
209. Lloyd, D. P. C., *J. Neurophysiol.*, **6**, 111-20 (1943)
210. Lloyd, D. P. C., *J. Neurophysiol.*, **6**, 293-315 (1943)
211. Lloyd, D. P. C., *J. Neurophysiol.*, **6**, 317-26 (1943)
212. Lloyd, D. P. C., *J. Gen. Physiol.*, **33**, 147-70 (1949)
213. Lloyd, D. P. C., *J. Gen. Physiol.*, **41**, 297-306 (1957)
214. Loewenstein, W. R., and Altamirano-Orrego, R., *Nature*, **181**, 124-25 (1958)
215. Loewenstein, W. R., and Rathkamp, R., *Science*, **127**, 341 (1958)
216. Lombroso, C. T., and Merlis, J. K., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 301-8 (1957)
217. Lorente de N6, R., *Arch. Neurol. Psychiat.*, **30**, 245-91 (1933)
218. Lorente de N6, R., *J. Neurophysiol.*, **1**, 207-44 (1938)
219. Lorente de N6, R., in *Physiology of the Nervous System*, 3rd ed., Chap. 15 (Fulton, J. F., Ed., Oxford University Press, New York and London, 1248 pp., 1949)
220. Lüthy, F., *Conf. neurol.*, **17**, 82-94 (1957)
221. McIntyre, A. K., *Nature*, **168**, 168-69 (1951)
222. MacLean, P. D., *Arch. Neurol. Psychiat.*, **78**, 113-27 (1957)
223. MacLean, P. D., *Arch. Neurol. Psychiat.*, **78**, 128-42 (1957)
224. MacNichol, E. F., Macpherson, L., Svaetichin, G., and Krattenmacher, W., *Symposium on Visual Problems of Colour* (National Physical Laboratory, Teddington, Middlesex, Engl., 1957)
225. Mandell, A. J., and Bach, L. M. N., *Am. J. Physiol.*, **190**, 330-32 (1957)
226. Matthews, P. B. C., *Proc. Physiol. Soc.*, **138**, 28-29 (1957)
227. Matthews, P. B. C., *J. Physiol. (London)*, **140**, 408-20 (1958)
228. Matthews, P. B. C., Phillips, C. G., and Rushworth, G., *Quart. J. Exptl. Physiol.*, **43**, 38-52 (1958)
229. Matthews, P. B. C., and Rushworth, G., *Proc. Physiol. Soc.*, **131**, 30-31 (1956)
230. Matthews, P. B. C., and Rushworth, G., *J. Physiol. (London)*, **135**, 245-62 (1957)

231. Matthews, P. B. C., and Rushworth, G., *J. Physiol. (London)*, **135**, 263-69 (1957)
232. Matthews, P. B. C., and Rushworth, G., *J. Physiol. (London)*, **140**, 421-26 (1958)
233. Melzack, R., and Hangen, F. P., *Am. J. Physiol.*, **190**, 570-74 (1957)
234. Meyer, J. S., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 83-100 (1957)
235. Meyer, J. S., and Denny-Brown, D., *Neurology*, **7**, 447-58 (1957)
236. Meyer, J. S., and Hunter, J., *J. Neurosurg.*, **14**, 210-27 (1957)
237. de Molina, A. F., and Gray, J. A. B., *J. Physiol. (London)*, **137**, 126-40 (1957)
238. de Molina, A. F., Gray, J. A. B., and Palmer, J. F., *J. Physiol. (London)*, **141**, 169-76 (1958)
239. Morlica, A., Moruzzi, G., and Naquet, R., *Electroencephalog. and Clin. Neurophysiol.*, **5**, 571 (1953)
240. Morin, F., *Am. J. Physiol.*, **183**, 245-52 (1955)
241. Morin, F., and Catalano, J. V., *J. Comp. Neurol.*, **103**, 17-32 (1955)
242. Morin, F., Catalano, J. V., and Lamarche, G., *Am. J. Physiol.*, **188**, 263-73 (1957)
243. Morin, F., Lamarche, G., and Ostrowski, A. Z., *Am. J. Physiol.*, **189**, 401-6 (1957)
244. Morin, F., Lindner, D., and Catalano, J., *Am. J. Physiol.*, **188**, 257-62 (1957)
245. Morin, F., and Thomas, L. M., *Anat. Record*, **121**, 344 (1955)
246. Morrell, R. M., *Nature*, **180**, 709 (1957)
247. Moruzzi, G., and Pompeiano, O., *Rev. arch. ital. biol.*, **95**, 31-55 (1957)
248. Moruzzi, G., and Pompeiano, O., *J. Comp. Neurol.*, **107**, 1-25 (1957)
249. Motokawa, K., Oikawa, T., and Tasaki, K., *J. Neurophysiol.*, **20**, 186-99 (1957)
250. Mountcastle, V. B., *Ann. Rev. Physiol.*, **20**, 471-508 (1958)
251. Mountcastle, V. B., *J. Neurophysiol.*, **20**, 408-34 (1957)
252. Mountcastle, V. B., Davies, P. W., and Berman, A. L., *J. Neurophysiol.*, **20**, 374-407 (1957)
253. Mozell, M. M., *J. Neurophysiol.*, **21**, 183-96 (1958)
254. Murray, J. G., *J. Physiol. (London)*, **135**, 206-12 (1957)
255. Murray, R. W., *Nature*, **179**, 106-7 (1957)
256. Nakayama, T., *Japan. J. Physiol.*, **7**, 99-112 (1957)
257. Oscarsson, O., *Acta Physiol. Scand.*, **40**, 222-31 (1957)
258. Oscarsson, O., *Acta Physiol. Scand.*, **42**, Suppl. 146, 1-107 (1957)
259. Oswald-Cruz, E., and Tsouladzé, S., *J. physiol. et pathol. gén.*, **49**, 327-29 (1957)
260. Papez, J. W., *Arch. Neurol. Psychiat.*, **38**, 725-43 (1937)
261. Perl, E. R., *Am. J. Physiol.*, **188**, 609-15 (1957)
262. Perl, E. R., *J. Neurophysiol.*, **21**, 101-12 (1958)
263. Pompeiano, O., and Brodal, A., *Rev. arch. ital. biol.*, **95**, 166-95 (1957)
264. Powell, E. W., Haggart, J., Goodfellow, E., and Niemer, W. T., *Neurology*, **7**, 689-96 (1957)
265. Proctor, L. D., Knighton, R. S., and Churchill, J. A., *Neurology*, **7**, 193-203 (1957)
266. Purpura, D. P., Girado, M., and Grundfest, H., *Proc. Soc. Exptl. Biol. Med.*, **95**, 791-96 (1957)
267. Purpura, D. P., Girado, M., and Grundfest, H., *Science*, **125**, 1200-2 (1957)
268. Purpura, D. P., and Grundfest, H., *J. Neurophysiol.*, **19**, 573-95 (1956)
269. Purpura, D. P., and Grundfest, H., *J. Neurophysiol.*, **20**, 494-522 (1957)

270. Rexed, B., and Brodal, A., *J. Neurophysiol.*, **14**, 399-407 (1951)
271. dal Ri, H., and Schaefer, K. P., *Arch. ges. Physiol.*, **265**, 125-37 (1957)
272. Rioch, D. McK., in *Symposium on Brain Mechanism and Drug Action*, 142-47 (Fields, W. S., Ed., Charles C Thomas, Publisher, Springfield, Ill., 147 pp., 1957)
273. Rosenzweig, M. R., *J. Comp. and Physiol. Psychol.*, **47**, 269-76 (1954)
274. Rosenzweig, M. R., and Sutton, D., *J. Neurophysiol.*, **21**, 17-23 (1958)
275. Rossi, G. F., and Brodal, A., *Arch. Neurol. Psychiat.*, **78**, 439-53 (1957)
276. Rossi, G. F., and Zanchetti, A., *Rev. arch. ital. biol.*, **95**, 199-438 (1957)
277. Rothballer, A. B., *Electroencephalog. and Clin. Neurophysiol.*, **8**, 603-21 (1956)
278. Rothballer, A. B., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 409-17 (1957)
279. Schoolman, A., and Delgado, J. M. R., *J. Neurophysiol.*, **21**, 1-16 (1958)
280. Shealy, N. C., and Peele, T. L., *J. Neurophysiol.*, **20**, 125-39 (1957)
281. Sigg, E., Ochs, S., and Gerard, R. W., *Am. J. Physiol.*, **183**, 419-26 (1955)
282. Sigg, E. B., and Schneider, J. A., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 419-26 (1957)
283. Snider, R. S., McCulloch, W. S., and Magoun, H. W., *J. Neurophysiol.*, **12**, 325-34 (1949)
284. Sperry, R. W., *J. Neurophysiol.*, **10**, 275-94 (1947)
285. Sperry, R. W., Minor, N., and Myers, R. E., *J. Comp. and Physiol. Psychol.*, **48**, 50-8 (1955)
286. Spiegel, E. A., Szekely, E. G., and Baker, W. W., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 291-99 (1957)
287. Sprague, J. M., and Chambers, W. W., *J. Neurophysiol.*, **16**, 451-63 (1953)
288. Sprague, J. M., and Chambers, W. W., *Am. J. Physiol.*, **176**, 52-64 (1954)
289. Stamm, J. S., and Sperry, R. W., *J. Comp. and Physiol. Psychol.*, **50**, 138-43 (1957)
290. Suda, I., Koizumi, K., and Brooks, C. McC., *Am. J. Physiol.*, **189**, 373-80 (1957)
291. Suda, I., Koizumi, K., and Brooks, C. McC., *J. Neurophysiol.*, **21**, 113-22 (1958)
292. Sugar, O., Chusid, J. G., and French, J. D., *J. Neuropathol. Exptl. Neurol.*, **7**, 182-89 (1948)
293. Svaetichin, G., *Acta Physiol. Scand.*, **29**, Suppl. 106, 565-600 (1953)
294. Szabo, T., and Albe-Fessard, D., *J. physiol. (Paris)*, **46**, 528-31 (1954)
295. Szekely, E. G., *Conf. neurol.*, **17**, 243-49 (1957)
296. Szentagothai, J., *J. Neurophysiol.*, **11**, 445-54 (1948)
297. Talbot, S. A., and Kuffler, S. W., *J. Opt. Soc. Am.*, **42**, 931-36 (1952)
298. Thomas, D. M., Kaufman, R. P., Sprague, J. M., and Chambers, W. W., *J. Anat.*, **90**, 371-84 (1956)
299. Tomita, T., *Japan. J. Physiol.*, **7**, 80-85 (1957)
300. Torvik, A., and Brodal, A., *Anat. Record*, **128**, 113-37 (1957)
301. Trincker, D., *Arch. ges. Physiol.*, **264**, 351-82 (1957)
302. Tschirgi, R. D., Inanaga, K., Taylor, J. L., Walker, R. M., and Sonnenschein, R. R., *Am. J. Physiol.*, **190**, 557-62 (1957)
303. Wagman, I. H., Werman, R., Feldman, D. S., Sugarman, L., and Krieger, H. P., *J. Neuropathol. Exptl. Neurol.*, **16**, 269-77 (1957)
304. Wallberg, F., *Brain*, **80**, 273-87 (1957)
305. Wall, P. D., and Johnson, A. R., *J. Neurophysiol.*, **21**, 148-58 (1958)

- 306. Welker, W. I., Benjamin, R. M., Miles, R. C., and Woolsey, C. N., *J. Neurophysiol.*, **20**, 347-64 (1957)
- 307. Whitfield, I. C., *Electroencephalog. and Clin. Neurophysiol.*, **9**, 35-42 (1957)
- 308. Whitteridge, D., *Quart. J. Exptl. Physiol.*, **40**, 331-36 (1955)
- 309. de Vito, R. V., Brusa, A., and Arduini, A., *J. Neurophysiol.*, **19**, 241-53 (1956)
- 310. Woolsey, C. N., Settlage, P. H., Meyer, D. R., Sencer, W., Hamue, T. P., and Travis, A. M., *Research Publ. Assoc. Research Nervous Mental Disease*, **30**, 238-64 (1952)
- 311. Zotterman, Y., *J. Physiol. (London)*, **95**, 1-28 (1939)

VISCERAL FUNCTIONS OF THE NERVOUS SYSTEM¹

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INTRODUCTION

Interpretations of the scope of this chapter have varied considerably in previous years, usually in accordance with the particular long-term research orientation of the reviewer. In keeping with this tendency, the present review will be organized primarily in terms of the participation of the central nervous system in the regulation of endocrine, autonomic and, consequently, metabolic functions. It appears in general that the importance of central nervous system influences upon visceral functions, particularly endocrine regulation, has not been fully appreciated, probably for a number of reasons.

In the first place, there has been the rather well-established general impression, supported by experimental data, that visceral functions are largely autonomous and responsive primarily to metabolic needs. The existence of these plausible concepts of self-regulation has possibly created resistance to the idea that an additional set of regulatory influences of considerable practical importance might be exerted upon visceral functions by the central nervous system.

Probably a more important reason, however, has been the crucial matter of experimental approaches and methods. It is clear, first of all, that a comprehensive experimental approach to neural regulation of visceral function can only be achieved by the pooling of skills and viewpoints of a broad range of scientific disciplines. These combined approaches must permit, on the one hand, an analysis of central nervous system mechanisms (by the techniques of neuroanatomy, neurophysiology, neuropharmacology, neurochemistry, experimental and clinical psychology, and psychiatry) and, on the other hand, the analysis of specific visceral functions (by the techniques of chemistry, physiology, pathology, immunology, and internal medicine). Impetus for collaboration between various combinations of disciplines within these two general groups has come from both directions. Many behavioral scientists have come to view hopefully the measurements of visceral function, which are relatively objective and quantitative, as a major approach to the experimental analysis of emotional states, which have been extraordinarily difficult to evaluate by psychological techniques alone. Similarly, physiologists and internists have become increasingly aware of the necessity for a greater understanding of the impact of emotional disturbances upon bodily function and of the possible participation of emotional factors in the development of disease.

¹ The survey of literature pertaining to this review was concluded June, 1958.

Within the past five to ten years, substantial new methodological developments have emerged in many fields and have made possible a new order of directness and accuracy in psychophysiological research. An attempt will be made in this review to point out some of these developments and the new, relatively unexplored areas which have been opened to investigation as a result. In spite of these encouraging advances, methods remain a major limiting factor delaying progress in this field, and attention will be given to some of the areas where improved methods are urgently needed.

PSYCHOLOGICAL CORRELATES OF VISCERAL FUNCTIONS

In general, one of the most encouraging recent developments in this difficult field of working out relationships between psychological and physiological processes has been the development and successful use of new approaches and methods for the systematic experimental analysis of emotional reactions in both man and laboratory animals. The impact of these new methods is already evident in this review of recent work.

Studies of environmental change.—A great deal of attention has been concentrated upon the problem of emotional influences on the anterior pituitary-adrenal cortical system since its role as a general adaptive mechanism, responsive to a wide variety of physical stimuli, has been confirmed. One of the clearest relationships to emerge recently in this field has been that between situations of novel environmental change and pituitary-adrenal cortical activity. An impressive array of evidence has indicated marked plasma or urinary corticosteroid elevations in the following situations: the first handling and venipuncture in monkeys (1); the first move from home cage to experimental cage in monkeys (2); the transfer to an experimental restraining chair in monkeys (1); admission to hospital for elective surgery in patients (3); transfer of psychiatric patients from ward into an unfamiliar laboratory on an initial, or pre-experimental day (4); period following transport of cattle to a veterinary college animal quarters (5); transfer of cattle and sheep from loose box to urine collection crate and commencement of urine collection and handling by strangers (5); and the journey to hospital laboratory in "normal" control subjects (6). A remarkable feature in some of these observations has been the intensity and duration of the pituitary-adrenal cortical response following introduction into an unfamiliar environmental situation. Holcombe (5), in his extensive and careful observations of cattle and sheep, noted that the elevated excretion levels of "reducing corticoids" in cattle following transfer to his laboratory persisted in some animals as long as a month or more before stabilization at normal levels occurred. After a brief 2 to 5 min. period of catching, restraining, and venipuncture in monkeys, Mason *et al.* (1) observed that marked plasma 17-hydroxycorticosteroid (17-OH-CS) elevations persisted for several hours in some animals. In the same study it was observed that adaptation occurred with remarkable rapidity, as evidenced by the absence of corticosteroid elevations in the second and subsequent experiments. Sabshin *et al.* (4) and Persky *et al.* (7), in multidisciplinary studies in patients with anxiety, observed that plasma

and urinary 17-OH-CS levels as well as anxiety were as high or higher in subjects on an initial or pre-experimental day than on subsequent days when attempts were made to produce emotional distress experimentally. The important implications of these findings in relation to psychosomatic research methodology are discussed as well as the need for intensive, systematic research on the psychophysiology of novelty and ambiguity (4).

These findings of environmental influences call for some re-evaluation of the meaning of "normal" levels of pituitary-adrenal cortical activity. In a recent study, urinary 17-OH-CS measurements in "normal" monkeys kept in a laboratory where there was routine biochemical research going on five days a week, but not on weekends, showed a striking weekly rhythm in 17-OH-CS excretion. Levels were highest on Monday, rather stable between Tuesday and Friday, but dropped about 30 per cent lower on Saturday and Sunday (1). Variables such as lighting, room temperature, and feeding were approximately constant seven days a week, but the major variable appeared to be the absence of people and activity on weekends. These findings would seem to indicate that even the relatively subtle interactions of animals with the everyday environment may be reflected sensitively in the level of pituitary-adrenal cortical activity and that a concept of "basal" rather than "normal" levels for this system might be a useful one. Such observations as those enumerated above suggest strongly that certain environmental factors, probably associated with emotional arousal, have substantial influences on ACTH regulation, but further characterization of these emotional factors must depend upon more exact techniques of behavioral analysis.

Studies of conditioned emotional disturbances.—In the field of experimental psychology, it has become clear that operant conditioning methods have vast and largely unexplored potentialities for both the production and the measurement of emotional responses in animals. A procedure for producing a conditioned emotional response often referred to as conditioned "anxiety" or "fear", or simply, "CER," was first reported by Estes & Skinner (8) and later modified and applied by Brady & Hunt (9). It is concerned with the suppressing effect of "anticipated" pain upon stable lever pressing behavior in animals working for food or water rewards. Sidman (10) has described a conditioned avoidance procedure in which animals are trained to press a lever in order to avoid a brief shock which would otherwise be delivered automatically at regular intervals. A conditioned punishment procedure (11, 12) is closely related to, but somewhat different from the above procedures. Preliminary experience with some more complicated multiple schedule procedures indicates that it may be possible to structure experimental situations to include elements of uncertainty or ambiguity, as well as threat of noxious stimulus (13).

Some of the early interdisciplinary applications of these methods were concerned with the role of hormones in the regulation of behavior, rather than the converse. Solomon & Wynne (14) studied the development of avoidance conditioning in sympathectomized dogs, finding that such animals could establish avoidance responses although learning took significantly

longer and extinction proceeded more rapidly than in normal controls. In an interesting preliminary report, Mirsky *et al.* (15) described some studies on the effects of ACTH on the normal course of avoidance conditioning and extinction as well as its effects on a conditioned "fear" response in monkeys. Monkeys given ACTH during the period of acquisition of a conditioned "fear" response subsequently showed markedly less reaction to the conditioned "fear" stimulus. ACTH-treated monkeys also showed exceptionally rapid extinction of a conditioned avoidance response. These two studies are presented as examples of promising experimental approaches to an important phase of neuroendocrinological research, i.e., the study of "feedback" effects of hormones on the central nervous system itself which must certainly be taken into account in the total picture of visceral regulation.

The changes in plasma 17-OH-CS levels associated with various conditioning procedures in the rhesus monkey have been recently reported by Mason *et al.* (2). Both a conditioned "anxiety" or "fear" situation (anticipation of pain) and a conditioned avoidance situation (avoidance of pain) were associated with similar marked increases in plasma 17-OH-CS levels (approx. 20 to 25 μ g. per cent per hr.) which were as great as those observed following injection of a large dose of ACTH (16 mg. per kg.). It was also shown that variations in the conditions of stimulus duration and frequency in the conditioned "fear" situation were associated with variations in the intensity of the hormone response. By contrast, normal animals and animals which were pressing levers for food rewards on various schedules of reinforcement, including a 100:1 fixed ratio (100 presses required for 1 reward), did not show plasma 17-OH-CS elevations during sessions of comparable duration. The plasma 17-OH-CS responses associated with conditioned fear and conditioned avoidance persisted relatively unchanged for months when the animals were studied once weekly. Additional data confirming the intimate relationships between emotional disturbance and the corticosteroid response were revealed in another study (16) in which there were strikingly close correlations between the disappearance of the behavioral and that of hormonal responses to a conditioned "fear" stimulus in monkeys given repeated doses of reserpine.

The refinement of a chair-type apparatus for the gentle, long-term restraint of monkeys offers possibilities for many types of experimental techniques to be explored newly in the conscious primate, as discussed in a recent report (17). Using this chair, investigators have found it possible to make concurrent measurements of plasma epinephrine, norepinephrine, and 17-OH-CS levels during conditioned emotional disturbances in monkeys (13). Blood samples are obtained unobtrusively through a long polyethylene catheter inserted into the right atrium and exteriorized at the top of the head so that the animal is unable to reach it while in the chair. Two types of hormone response patterns were observed in stressful situations. In conditioned fear, avoidance, and punishment situations, a pattern of increased plasma 17-OH-CS and norepinephrine levels occurs, with little or no change in plasma epinephrine; in other more complex situations probably involving

considerable uncertainty, ambiguity, or novelty as well as threat of a noxious stimulus, marked plasma epinephrine as well as norepinephrine and 17-OH-CS elevations occurred (13).

Porter *et al.* (18) and Brady *et al.* (19) have reported a high incidence of chronic peptic ulceration in monkeys which are subjected to frequent emotional conditioning sessions for prolonged periods. In a series of 19 autopsies of monkeys on chronic programs of various disturbing conditioning procedures, 11 animals showed gastrointestinal disease, including five with duodenal ulceration and three with gastric erosion (18). Gross and microscopic evidence of chronicity was found in several cases. Such lesions are exceedingly uncommon in routine monkey necropsies (20). The preliminary phase of a systematic follow-up study (19) supports strongly that the psychological rather than the physical aspects of the experimental situation are critical for development of ulcers. This work also offers hope that chronic conditioning programs may be suitable for the development of a standard procedure for the experimental production of peptic ulcers in primates by predominantly psychological stress. Previous studies in rats have also indicated that experimental situations, believed to involve predominantly psychological stress such as approach-avoidance or fear situations, were associated with a high incidence of gastric lesions (21, 22). The possibly broader implications of the use of operant conditioning methods as a basis for research on a range of psychosomatic disorders outside the gastrointestinal tract have apparently not been explored yet.

Studies in psychiatric patients.—In clinical studies, a major limitation has been the need for experimental methods permitting a more systematic and explicit approach to the classification and rating of emotional states. In this respect two recent reports should have widespread effects on present and future psychosomatic research because of their introduction of improved concepts of objectivity and rigor in the making of psychological observations. The general characteristics of these improvements may be briefly enumerated as follows: (a) comprehensive observation; (b) systematic recording; (c) quantification; (d) cross-checking of observers; and (e) independence of behavioral and physiological data. In the first study, Engel *et al.* (23) carried out a long series of gastric secretory and psychological measurements in an infant with a gastric fistula and obtained impressive evidence that the total hydrochloric acid secretion rate was intimately integrated with total behavioral activity. Rising hydrochloric acid secretion rates were consistently associated with outgoing emotional states, either friendly or aggressive, which generally indicated active transactions of the infant with the environment. In the second, more recent, study reported by Hamburg *et al.* (24), two psychiatric observers watched subjects during a four-day period of laboratory study through a one-way vision mirror, keeping detailed observational notes and making independent ratings of the estimated intensity of anxiety, anger, and depression during various periods, including stressful interviews. A detailed analysis of the data, including a variety of interobserver comparisons, indicated a high degree of reliability of observa-

tions, suggesting that the phenomena under study although complex are reliably identifiable and quantifiable. Concurrent studies comparing these ratings of emotional states with adrenal cortical activity in anxious patients showed that the degrees of increase in anxiety, anger, depression, and a combined affect rating were significantly and linearly related to the change in plasma 17-OH-CS level (25). These data indicated that plasma 17-OH-CS increase was not associated with a single specific type but rather a variety of types of emotional arousal. A particularly striking increase in plasma 17-OH-CS levels occurred, however, when anxiety of a disintegrative nature developed. In related studies Board *et al.* (26) found that psychiatric patients within 24 hr. of hospital admission had elevated plasma 17-OH-CS levels in comparison with their own values seventeen days later and with normal controls. A smaller but significant elevation above normal in serum protein bound iodine was observed in the same patients on admission. Analysis of psychiatric subgroups in this study suggested that exceptionally high hormone levels were associated with severe depressive reactions. A follow-up study (27) confirming this observation indicated that the degree of plasma 17-OH-CS elevation was positively correlated with the clinically estimated severity of the depressive syndrome. In studies with intravenous corticotropin administration in anxious subjects, Persky (28) observed only slightly greater plasma 17-OH-CS responses, but substantially greater urinary 17-OH-CS excretion, than in normal subjects and suggests that this may support an interpretation of pituitary-adrenal cortical overactivity in patients with anxiety. Persky (29) also reports that injected hydrocortisone is removed faster from plasma in anxious subjects than in normals, although they excrete a smaller proportion of the hydrocortisone test load as dihydroxyacetone side chain compounds. This would seem to indicate abnormalities in metabolism, as well as in rate of secretion, of the adrenal cortical hormones. The value of multivariate techniques in establishing psychophysiological relationships was shown by Glickstein *et al.* (30) who demonstrated, in anxious patients studied over a four-day period, two distinct temporal heart rate patterns correlating with differences in personality and affective response in a psychological stress situation. Other encouraging recent reports dealing with techniques applied to the characterization or quantitation of emotional states in human subjects are those of Thaler *et al.* (31), Price *et al.* (3), and Gottschalk & Kaplan (32).

Recent studies in schizophrenic patients (33, 34) have in general not established the presence of any striking adrenal cortical or related metabolic alterations as suggested in some earlier work, at least when schizophrenics are taken as a gross diagnostic category without systematic attempts to evaluate affective or clinical state at the same time biochemical measurements are being made. Some other possible explanations for apparently conflicting results in this field are discussed by Romanoff *et al.* (33). Continued studies of blood ceruloplasmin levels in schizophrenics and other groups of patients (35 to 38) have also shown variable results and have offered little hope of establishing diagnostic or etiological significance to this substance.

Studies of stressful life situations.—One of the most productive approaches to the investigation of emotional states and associated physiological changes in human subjects has been the study of natural life situations generally considered to be particularly stressful or disturbing and which might be expected to elicit a high incidence of emotional reactions in a group of normal people. Most of these studies have been focussed upon anterior pituitary-adrenal cortical activity in anxiety-provoking situations, and several have employed biochemical measurements of adrenal cortical hormone levels in blood or urine. Hill and co-workers in Thorn's group (39) made extensive observations on the stress of collegiate crew racing. The importance of psychological factors in causing increased urinary 17-OH-CS excretion during race and time trial days was supported by the fact that normal corticoid output was observed on practice days which involved a comparable amount of physical exercise. Bliss *et al.* (40) included, in a rather broad survey of natural and experimentally designed situations involving emotional disturbance, the study of medical students prior to comprehensive examinations and observed plasma 17-OH-CS elevations to mean levels around 18 to 20 μg . per cent compared to about 13 μg per cent for normal subjects. In a recent study of patients prior to elective thoracic surgery, Price *et al.* (3) found mean plasma 17-OH-CS levels around 21 to 23 μg . per cent in subgroups with high clinical ratings of emotional distress. These values were in the same range as those observed in chronic organic disease and in patients from the third to the seventh day postoperatively, suggesting that plasma 17-OH-CS levels above 20 μg . per cent in the human may represent the range generally found in situations of chronic stress, either psychological or physiological in origin. Strong positive correlations were observed between plasma 17-OH-CS levels and four Rorschach ratings, particularly those indicating emotional arousal. This, together with other findings, led to the development of a rating of "discomfort-involvement", based upon an overall estimate of the individual's emotionality and especially the extent of emotional investment or active participation in the current situation. Such ratings correlated highly with plasma 17-OH-CS levels. Connell *et al.* (41) studied eight students who were undergoing for the second time a comprehensive, four-day, professional examination in which failure would cost a loss of at least one year, and perhaps force permanent abandonment, of study. The severity of the situation was further attested by the fact that only two of the eight passed the examination. In these subjects, substantial elevations in urinary 17-ketogenic steroids, but only slight increase in 17-ketosteroids, were observed. Of considerable interest is a report by Venning *et al.* (42) indicating that substantial increases in aldosterone, as well as 17-OH-CS, excretion occurred in students undergoing examinations as compared to the same individuals during a control period. Furthermore, a good correlation was found between the degree of aldosterone increase and the degree of emotional disturbance, as estimated by means of interview and self-rating procedures.

In studies on members of aircraft crews after 15-hr. periods of flight,

Jones (43) found that secretory and motor responses of the stomach to a standard test meal were more marked than after corresponding rest periods. The rate of uropepsinogen excretion was also higher during 24-hr. periods which included a 15-hr. flight than during corresponding periods without flight.

In a study reported by Yessler *et al.* (44), blood pepsinogen levels at the time of induction into the army were correlated with the subsequent development of peptic ulcer during a two-year period of army service. Out of 2031 inductees, there was evidence of peptic ulcer development in 14 cases and, of these, 10 subjects fell within a "hyper-secretor" category, defined as those with blood pepsinogen levels greater than one standard deviation above the mean. In an earlier phase of this study, Weiner *et al.* (45) reported high correlation between the blood pepsinogen levels in these inductees and specific personality characteristics, as determined largely on the basis of projective test results.

PHYSIOLOGICAL ASPECTS OF THE REGULATION OF VISCERAL FUNCTIONS

Active investigation continues on the physical stimuli and the physiological factors, both neural and humoral, concerned in the regulation of visceral functions. Research in regulation of endocrine secretion has clearly been stimulated by the recent availability of several greatly improved hormone assay methods involving biochemical characterization of relatively purified chromatographic fractions. It appears, indeed, increasingly hopeful that the concurrent measurement in blood and urine of a variety of hormones representing each major endocrine system, which would permit observations of relative changes in secretory patterns as well as of absolute levels of specific hormones, may be relatively near at hand if biochemical methodologists continue to be attracted to this field. From the abundance of data indicating complex interaction and interdependence of many hormones at the metabolic level, as well as the possibility of humoral "feedback" effects on the secretory mechanisms themselves, it would seem that a comprehensive understanding of endocrine regulation must include consideration of balances in hormone secretion patterns as well as study of isolated endocrine systems. Several limited attempts in this direction during the past year (13, 26, 41, 42) justify further interest in this approach.

Studies of the pituitary-adrenal cortical system.—The response of the pituitary-adrenal cortical system to trauma has received considerable attention in studies on surgical patients. Helmreich *et al.* (46) found that a variety of major surgical procedures produced elevations in free plasma 17-OH-CS levels as great as those following ACTH, while there was considerable variation in conjugated plasma 17-OH-CS responses. Viikari & Thomasson (47) observed plasma 17-OH-CS elevations in a range between 30 to 50 μ g. per cent following abdominal and thoracic operations. Weichselbaum *et al.* (48) showed elevations of free and conjugated plasma Δ^4 -3-ketocorticosteroids, believed to represent potentially biological active corticosteroids, in patients after gastrectomy and burns. The duration and magnitude of increased

urinary 17-OH-CS excretion in patients with soft tissue and bone trauma were observed by Moore *et al.* (49) to correlate closely with the estimated severity of trauma and with nitrogen loss. Increased urinary 17-OH-CS excretion following surgery was also reported by Helmreich *et al.* (46) and by Reece *et al.* (50), the latter observing that elevations after inguinal hernioplasty were less than those following gastrectomy. In a study of some possible causes for the variations in plasma and urinary 17-OH-CS responses to surgery, Edwards *et al.* (51) reported that neither overreplacement of blood loss nor intravenous injection of testosterone appreciably affected the elevated mean urinary 17-OH-CS excretion during the first four postoperative days.

Hypoxia, equivalent to 14,000 ft. altitude, environmental temperature of 50°C., or both hypoxia and heat in combination did not induce elevations in plasma ACTH or 17-OH-CS levels within 45 min. in human subjects (52). Longer exposures produced delayed plasma 17-OH-CS elevations in some subjects. In a single subject studied for 134 days, winter to summer, MacFarlane & Robinson (53) reported decreased urinary excretion of 17-ketogenic steroids and 17-ketosteroids during the summer and during artificial heating.

Appleby & Norymberski (54) and Little *et al.* (55) have confirmed the gradual elevations in plasma 17-OH-CS levels occurring during pregnancy in human subjects as well as the sharp increases associated with delivery. A subgroup, the 21-deoxyketols, rose much more steeply than total urinary 17-hydroxycorticosteroids (54). Birke *et al.* (56) also observed elevated plasma 17-OH-CS levels during pregnancy and found slight increases in total urinary 17-ketosteroids, particularly the 11-oxygenated 17-ketosteroids. Androsterone and etiocholanolone were depressed. Wexler *et al.* (57) report evidence that Piromen, a bacterial polysaccharide, produces a hypophysial-mediated adrenal cortical response in the rat not accompanied by the usual antianabolic changes associated with ACTH release, and postulate the release of other anterior pituitary trophic hormones by this agent. Marks *et al.* (58) have obtained evidence that insulin increases 17-OH-CS excretion in studies of schizophrenic patients on insulin coma therapy. Jakobson (59), in a study of patients with hyperthyroidism, reports significant elevations in urinary 17-OH-CS and 17-ketogenic steroid excretion with a return to normal levels as soon as a euthyroid state is reached following treatment.

The question of the neurohumoral agent linking the hypothalamus and the ACTH-secreting cells of the anterior pituitary gland has received considerable attention. Clayton *et al.* (60) have shown that fraction D Δ , a nonpressor material extracted from protopituitrin and known to stimulate ACTH release *in vitro*, produced plasma 17-OH-CS elevations in patients during 4-hr. infusions or after intravenous injection. McCann (61) reports that pitressin evokes decreases in adrenal ascorbic acid in rats with hypothalamic lesions, pentobarbital-morphine anesthesia, or administration of hydrocortisone acetate, all three of which block stress-induced ACTH release. De Wied (62) found that a nonsaponifiable lipid extract of bovine

posterior hypothalamus caused a decrease in adrenal ascorbic acid in rats which could be blocked by hypophysectomy or by a combination of pentobarbital and chlorpromazine. On the other hand, McDonald *et al.* (63) conclude that in normal human subjects endogenous ADH release may occur without evidence of increased ACTH secretion and that ACTH release may occur without increased ADH secretion, based upon simultaneous measurements of plasma 17-OH-CS levels and urine osmolality in various test situations. Shapiro *et al.* (64) also concluded that physiological conditions which affect mobilization of ADH do not necessarily induce parallel alterations in ACTH secretion as judged by urinary 17-OH-CS excretion in both hydrated and dehydrated guinea pigs. In another study Shapiro *et al.* (65) present evidence that one mechanism whereby high blood levels of adrenal cortical hormones inhibit ACTH release during stress is through action on a cerebral (presumably hypothalamic) structure. By means of timed hypophysectomy in the rat, Long & Bonnycastle (66) determined that ACTH discharge, as judged by adrenal ascorbic acid depression, is initiated with great rapidity after unilateral adrenalectomy, with an initial phase lasting about five minutes followed later by a second period of increased secretion that is much more prolonged. They conclude that this is evidence for the participation of both neurohumoral and humoral mechanisms in the regulation of ACTH secretion.

Although reliable chromatographic biochemical methods for plasma and urinary adrenal cortical hormone determinations are well established, several pertinent new developments in methods have been reported (67, 68, 69).

Studies of aldosterone secretion.—Several groups of workers have been intensively investigating the interesting problem of the factors affecting aldosterone secretion. Several recent findings would appear to support the earlier work of Rauschkolb & Farrell (70, 71) which indicated that the diencephalon is involved in aldosterone regulation. Extracts of beef diencephalon produce increases in adrenal vein aldosterone output averaging 70 per cent above that of controls, according to Farrell *et al.* (72). Davis *et al.* (73) obtained confirmatory evidence that aldosterone secretion is not solely dependent upon the pituitary by observing that aldosterone excretion, sodium retention, and ascites can occur in hypophysectomized dogs after subsequent surgical constriction of the inferior vena cava. Casey *et al.* (74) observed that maximum aldosterone excretion occurred early after surgical operations and that it did not persist throughout the period of positive sodium balance in surgical patients. They discuss evidence indicating that a central nervous system mechanism is probably involved in the aldosterone response following surgery.

Some controversy has arisen concerning the possible role of aldosterone in the initiation of sodium and water retention in congestive heart failure. In two studies dealing with experimental congestive heart failure in dogs, no abnormalities in aldosterone secretion were observed either in adrenal venous output (75) or in urinary excretion (76). In other studies in dogs with

congestive failure, however, increases in both adrenal venous blood (77) and urinary excretion (78) have been observed. The reasons for these discrepancies are not yet apparent. In a clinical study, Bartter *et al.* (79) found that significant changes in aldosterone excretion could be effected by induced changes in body fluid volume, independent of changes in extracellular or intracellular ion or water concentrations.

Orti *et al.* (80) demonstrated the presence of a substance in urine of both intact and adrenalectomized rats deprived of salt which, when injected into intact hydrated rats, caused sodium retention and increased aldosterone excretion in the feces. Laidlaw *et al.* (81) have confirmed that aldosterone excretion is elevated during pregnancy, finding mean levels of 47 $\mu\text{g.}$ per day in the third trimester, compared with a normal level of 10 $\mu\text{g.}$ per day. Baulieu *et al.* (82) found no urinary aldosterone in a pregnant woman with Addison's disease and concluded that the placenta does not produce aldosterone. Although most of the reported research is based upon bioassay measurement of aldosterone, additional work has been reported recently on the development of completely biochemical procedures (83, 84).

Studies of catechol amine secretion.—Much needed reinvestigation of the regulation of both epinephrine and norepinephrine secretion has continued, largely with recently developed sensitive biochemical methods for catecholamine assay. In an extensive study on 102 surgical patients, Halme *et al.* (85) found marked increases in mean urinary epinephrine, norepinephrine, and 17-OH-CS excretion but no significant rise in excretion of 17-ketosteroids. Watts & Bragg (86) demonstrated increases in plasma epinephrine levels from less than 1.0 $\mu\text{g.}$ per l. to 12.5 $\mu\text{g.}$ per l. during hemorrhagic hypotension with automatic reinfusion of blood at 40 mm. Hg in dogs. Substantial epinephrine elevations were maintained for about 90 min., levels returning to 5 to 7 $\mu\text{g.}$ per l. during reinfusion. Manger *et al.* (87) found no change in plasma pressor amines with 18 per cent blood volume loss caused by hemorrhage, but observed changes from 1.0 to 7.8 $\mu\text{g.}$ per l. of epinephrine and from 2.5 to 3.6 $\mu\text{g.}$ per l. of norepinephrine in dogs with 33 per cent blood volume loss. In sensitized dogs showing anaphylactic shock when given egg white intravenously, mean plasma epinephrine elevations from 0.7 to 7.7 $\mu\text{g.}$ per l. were observed, with norepinephrine elevations occurring in some animals but not in others (87). Electroconvulsive shock has been reported by Griswold (88) to produce substantial but transient elevations in plasma epinephrine and norepinephrine levels in rats and in human subjects. Significant depression of the norepinephrine response in humans after a series of electroshock treatments was also observed. Gray & Beetham (89) reported marked increases in plasma norepinephrine levels after acute and chronic muscular work, but wide variation was observed in epinephrine responses. Goldfien *et al.* (90) found that insulin administration produced a marked increase in epinephrine levels, and a much less marked increase in norepinephrine levels in adrenal venous blood and peripheral blood of anesthetized dogs. Concurrent glucose infusion rapidly lowered the elevated secretion

rate of both epinephrine and norepinephrine, suggesting that hypoglycemia was the stimulus for elevated catecholamine secretion under these experimental conditions.

Some controversy continues with regard to the reliability of biochemical methods for plasma epinephrine and norepinephrine determinations, but an impressively increasing number of laboratories are reporting confidence in the Weil-Malherbe and Bone procedure, or its modifications, as evidenced in the work reviewed above. Some pertinent methodological reports in this field have recently appeared (91 to 95).

Studies of other endocrine systems.—Extremely scant data have appeared on the regulation of secretion in the remaining endocrine systems. Schwartz & Roberts (96) found consistent elevations in thyroid activity, as evidenced by serum protein bound iodine levels, within 3 hr. after major surgery is performed and also recorded levels below preoperative values 24 hr. postoperatively. Further improvements in thyroid hormone assay techniques, including methods for detection of tri-iodothyronine in plasma (97, 98), open the way for additional investigations of the regulation of thyroid secretion. A review by Thorn (99) covering many aspects of mammalian antidiuretic hormone physiology, including regulatory factors, has appeared recently. Some evidence for the separate release of oxytocin has been presented by Theobald (100) who found that rapid intravenous injection of a liter of 2.5 per cent saline solution caused no uterine contractions in patients and that suckling causes no, or very little, antidiuresis. Buchborn (101), using a bioassay in the toad, found that plasma antidiuretic hormone levels in healthy humans showed close correlations with serum osmolality. Evidence that the antidiuretic effect accompanying laboratory-induced motion sickness may be on a posterior pituitary basis has been reported by Taylor *et al.* (102). In spite of steady improvements in biochemical methods for sex hormone determinations, both androgens (103, 104) and estrogens (105 to 109), application of these techniques remains largely limited to clinical usage, so that experimental data on the regulation of gonadal function, particularly with respect to environmental influences, are extremely sparse.

Perhaps the single greatest obstacle to greater progress in the study of neuroendocrine relationships has been the lack of suitable quantitative methods for the measurement of the concentrations in body fluids of the protein and peptide hormones, including those of both the anterior and posterior pituitary glands as well as insulin. In many cases adequate chromatographic procedures have been developed for separating and purifying these hormones, but more sensitive and specific techniques for characterization of the isolated material are apparently required. In view of the continuing, scattered reports on the detection of specific antibodies to purified hormone preparations (110, 111, 112), it appears possible that the application of newly developed immunochemical methods, in conjunction with biochemical techniques, to the problem of quantitative as well as qualitative assay of protein or peptide hormones is an approach worthy of serious exploration.

Studies of autonomic functions.—Some new information has been reported on the effects of physical stress or physiological influences on the

regulation of autonomic functions. Clemedson (113) has studied respiratory and circulatory vagal reflexes in rabbits exposed to high explosive shock waves. He found that the rapid, shallow breathing after detonation in normal animals was almost completely absent after cervical vagotomy or pulmonary vagal denervation and that cardiac standstill 1 to 3 sec. after detonation was common in normals and animals with lung vagus denervation, but rare in animals with bilateral cervical vagotomy. Baugh *et al.* (114) found that immersion of one arm in a 5°C. bath for one hour decreased plasma volume and increased hematocrit levels, and they noted slight differences in response between eskimo and white subjects. LeBlanc & Rosenberg (115) showed that the increased systolic arterial pressure response persists through eight repetitions of the cold pressor test but that the fall in arterial pressure below pretest values after removal of the hand from the cold bath gradually disappeared with repetition. This adaptation is believed to be on a systemic basis.

Hix (116) has reported further observations on factors influencing renal function in the conscious animal. He has demonstrated a ureterorenal reflex whereby unilateral irritation of the ureter causes diminished renal plasma flow and glomerular filtration rate. He discusses the possibility that its functional significance may be as a protective phenomenon. Berman & Rose (117) found no significant cardiovascular alterations associated with acute pressure changes within the bladder and renal pelves of intact anesthetized dogs, in marked contrast to the responses seen in reptilia and in amphibia. Distention of one uterine horn in conscious dogs produced no consistent effect on urinary loss of water, sodium, or potassium (118). Patients with idiopathic autonomic hypofunction manifested by postural hypotension hypohidrosis and impotence have enhanced ability to excrete sodium and solute free water in response to rapid sodium chloride infusion. It is suggested that well-developed autonomic vasoregulatory reflexes functioning to maintain circulatory homeostasis may result in man's relative inability to increase glomerular filtration rate and excretion of salt excesses rapidly (119).

Techniques for the measurement of arterial pressure by indirect methods (120) and for producing experimental hypertension (121) in the rhesus monkey have been reported. The problem of continuous recording of arterial blood pressure has been analyzed by Davis (122). Some recent techniques for study of cardiac function by intracardiac phonocardiography (123) and the use of crystal radiation detectors on the chest wall (124) have been described.

An extensive review of the subject of pepsinogen by Hirschowitz (125) includes discussion of regulatory influences on pepsinogen secretion. The confusion resulting from inadequate experimental study of the significance of common indices of pepsinogen secretion is analyzed (125). Most of the research with pepsinogen measurements has been heavily concentrated upon the peptic ulcer patient and very little is yet known of possible fluctuations which may occur in response to physical stresses or physiological disturbances. Soiva *et al.* (126) have recently reported progressive elevations of uropepsinogen excretion during pregnancy, with even greater rises in pa-

tients with toxemia, but it is believed that these changes may be related to the known adrenal cortical hyperfunction in pregnancy. Concurrent measurements of adrenal cortical and pepsinogen secretion in a variety of acute and chronic stress situations may lead to a better understanding of the relationships between the regulation of these two functions.

Another detailed review of interest deals with the subject of the mechanics and regulation of gastric emptying (127). Several reports pertinent to gastrointestinal methodology have appeared, concerned with motility measurements by a pressure-sensitive, radiotelemetry capsule (128), and recording of abdominal wall potentials (129). Transducer-measured intraluminal pressure recordings have been correlated with fluoroscopic measurements of motor activity (130, 131). Techniques for continuous recording of gastric contractions (132) and gastric secretion (133, 134) in the rat have been described.

In a study of physiological factors that regulate eating behavior, Smith & Duffy (135) found that hypertonic fluids, intraperitoneal injection of several sugars, and particularly intragastric bulk reduced eating in rats. Weiss (136) has studied effects of brief exposures to cold on food intake and concludes that the feeding schedule under observation is quite sensitive to changes in temperature and that there is a short latency of response to its effects.

Several interesting studies have been carried out on the mechanism of diurnal rhythms of certain visceral functions. A very broad review of the subject of diurnal rhythms in animals has been written by Harker (137). In a study of two groups of human subjects living in Spitzbergen, one group on a 21-hr. day, the other on a 27-hr. day, Lewis & Lobban (138) conclude that there is an intrinsic 24-hr. rhythm of water, potassium, and chloride excretion which persists in the majority of subjects through seven-week periods of abnormal time routines. Lewis & Lobban (139) also report evidence of dissociation of diurnal rhythms of body temperature and the excretory rhythms of water, potassium, and chlorides. In some instances there was even dissociation of potassium from water and chloride excretory rhythms. The authors conclude that more than one mechanism may control physiological diurnal rhythms in man and that evidence is strong that one of these mechanisms is central (139). Halberg *et al.* (140) found that hemidecortication in humans does not affect persistence of 24-hr. rhythms in rectal temperature and eosinophil count. Ferguson *et al.* (141) report that hypophysectomy caused hypothermia and some flattening of diurnal temperature rhythm in mice but that this was probably related to decreased activity of the animals. Iampietro *et al.* (142) observed little or no effect of diverse climates on diurnal patterns of rectal temperature in humans. Fasting alone, or fasting plus exercise did reduce or eliminate the normal elevation occurring between 8 a.m. to 8 p.m., although exercise when food intake was adequate did not alter the pattern. However, since complete 24-hr. observations were not made, it can not be concluded that diurnal temperature rhythm is completely abolished under these conditions. Studies in patients have indicated that reversal or leveling of the diurnal rhythm of plasma 17-OH-CS concentra-

tions is extremely uncommon, even under conditions of preoperative stress, and that some individuals show evidence of exaggeration of the diurnal rhythm under these conditions (3). It appears possible that the diurnal rhythm of visceral functions may reflect the balance between regulatory influences of basic importance, and that with its elucidation will come new information about the functional organization of the nervous system.

NEUROANATOMICAL ASPECTS OF THE REGULATION OF VISCERAL FUNCTIONS

It has been recognized for a long time that a variety of visceral functions are integrated within the central nervous system at the level of the caudal brain stem and the hypothalamus. Within recent years it has become clearer that this is true of endocrine as well as autonomic functions, although much remains to be done in defining possible localization of mechanisms governing specific visceral functions at these lower levels. The growing body of information indicating the marked influences of psychological and emotional factors upon visceral functions makes it more imperative that our search for the neural mechanisms underlying regulation of vegetative functions be extended to include studies of the central nervous system levels above the hypothalamus.

The limbic system.—In surveying the levels above the brain stem for structures which might be expected to exert modulatory influences upon the visceral functions represented in the hypothalamus, the limbic system must be placed high on the list of possibilities for at least two reasons. First there is good evidence, recently reviewed by Nauta (143) and Gloor (144), that limbic system structures, particularly the amygdaloid complex and the hippocampus, have substantial projections to the hypothalamus and reticular formation. Secondly there is a rapidly growing body of evidence, recently reviewed by Brady (145), that limbic structures are largely concerned with emotional phenomena. Background information regarding both conceptual and experimental aspects of limbic system function is presented by MacLean (146, 147), whose work and writings have been instrumental in the recent development of interest in this field.

The amygdaloid complex has been under intensive study since Schreiner & Kling's confirmation (148) of earlier work demonstrating striking changes in emotional, particularly sexual, behavior in animals with bilateral temporal lobectomy. Some confusion has developed in this field, however, with conflicting behavioral changes reported by various workers carrying out temporal lobe ablation studies. This problem has been discussed recently by Wood (149) who makes the logical suggestion that these differences may be a result of varying degrees of damage to the temporal lobe structures. In a study involving discrete electrocoagulation and stimulation of six distinct nuclear masses in the cat, Wood demonstrated that some of the component parts of the temporal lobe syndrome can be localized to definite nuclei of the amygdaloid complex. Discrete bilateral lesions of the lateral amygdaloid nucleus produced hypersexuality which was intensified if lesions were also present in the ventral claustrum. Bilateral lesions of the central amygdaloid nucleus resulted in increased food intake and aggressiveness (149). Kling &

Hutt (150) have shown that lesions in the ventromedial hypothalamus or mammillary bodies abolish the syndrome of altered behavior which follows removal of the amygdala and overlying cortex. Some observations of autonomic and emotional reactions associated with stimulation of the amygdala in cats have been reported by De Molina & Hunsperger (151) and Shealy & Peele (152). Wood *et al.* (153) present evidence that the corticomedial division of the amygdaloid complex is concerned with regulation of autonomic functions and that effects of the amygdala on arterial pressure and respiration are mediated through the ventromedial nucleus of the hypothalamus. Mason (154) has found that electrical stimulation of the amygdaloid complex through chronically implanted electrodes in conscious monkeys is associated with marked elevations of plasma 17-OH-CS levels (20 μ g. per cent per hr.) of the same order as those observed with hypothalamic stimulation or injection of very large doses of ACTH. Bunn & Everett (155) observed that, in rats with persistent estrus induced by continuous illumination, electrical stimulation of the amygdala through permanent bipolar electrodes produced ovulation in 5 out of 11 rats, suggesting that the LH-release apparatus may be influenced by neural elements higher than the hypothalamus. Sen & Anand (156) found marked increases in gastric secretory volume, acidity, and pepsin content as a result of electrical stimulation of the amygdaloid complex, the tip of the temporal lobe, and the posterior orbital surface of the frontal lobe in unanesthetized cats. Stimulation of the anterior cingulate and hippocampus did not produce any such increases.

Continuing his series of systematic brain stimulation studies in conscious goats, Andersson (157) found that electrical stimulation in the septal region produced cold defense reactions such as shivering, peripheral vasoconstriction, sometimes piloerection, and, in animals exposed to heat, an inhibition of polypneic panting. Prolonged stimulation in this region at room temperature caused elevations in rectal temperature up to 0.5°C. In a related study Andersson & Persson (158) found that polypnea, peripheral vasodilatation, inhibition of shivering, and pronounced hypothermia were maintained for long periods by continuous electrical stimulation of the pre-optic region when goats were kept in an environmental temperature of -6 to -7°C. Bond *et al.* (159) report that relatively small, bilateral, stereotaxic lesions in the septum, fornix, and anterior thalamus of cats elicited a variety of behavioral and visceral changes, including hypothermia, piloerection, hyperglycemia, and a high incidence of postoperative deaths.

MacLean (160) has reported a new technique permitting both chemical and electrical stimulation of limbic system components in the cat. His findings suggest that a neural system involving parts of the hippocampus, cingulate gyrus, and septum is involved in pleasure and grooming reactions and in certain aspects of sexual behavior (161). In other studies of cats and squirrel monkeys with carbachol-induced hippocampal seizures, he has demonstrated disruption of performance in animals trained in conditioned escape behavior (162). Conditioned cardiac and respiratory responses were also abolished or altered in animals with such seizures.

Some primarily behavioral studies of interest are those of the following

workers: Thomas & Otis (163) dealing with effects of hippocampal lesions on maze learning in rats; Weiskrantz & Wilson (164) on the effects of limbic system lesions on avoidance thresholds in monkeys; Mirsky *et al.* (165) describing temporary changes in behavior after cingulectomy in monkeys; and Alonso-de-Florida & Delgado (166) reporting lasting behavioral changes (documented by time-lapse photography) associated with prolonged stimulation of the amygdala in cats. In addition, two clinical reports confirm claims that memory deficits occur with lesions of the temporal lobe (167, 168).

In electroanatomical studies, Gloor (169) has extended and confirmed his earlier findings by demonstrating that subcortical structures fired by amygdaloid afterdischarges extend from the septal area back to the mesencephalon, with inclusion of the whole diencephalon. Pcwel *et al.* (170) reported that hypothalamic seizure activity in the cat can be elicited by stimulation of the amygdala, hippocampus, hippocampal gyrus, septum, and basal olfactory structures. They also describe evidence that different hippocampal areas affect different parts of the hypothalamus. In studies with the marsupial phalanger, Adey *et al.* (171) have described possible pathways between the entorhinal area and deep centers in the diencephalon. Davis (172) found increased locomotor activity in monkeys following caudate lesions but did not observe the gastrointestinal disorders reported by Rosvold & Delgado (173). Possible relationships of the basal ganglia to visceral functions are a problem worthy of further study.

The phenomenon of "self-stimulation" and its interesting implications with regard to limbic system functional organization cannot be covered here but is reviewed elsewhere in this volume. One of the most attractive summaries of current concepts of limbic system function is that of Gloor, who suggests that the limbic system, as a modulator of functional patterns integrated at the level of the hypothalamus and the brain stem tegmentum, may represent an important link between neocortex and hypothalamo-tegmental regions enabling the subcortically induced activities to adapt to the patterns organized by the neocortex (144). It appears likely that greater experimental emphasis on relationships between the limbic system and indices of endocrine and other visceral activity may speed the elucidation of limbic system function.

Hypothalamus and reticular formation.—An extensive anatomical study of the ascending pathways originating in the brain stem reticular formation has been reported by Nauta & Kuypers (174). This fundamental work should be of particular interest to the investigator of the regulation of visceral functions in that an extensive mesencephalic region is shown to project to the hypothalamus and even more rostral areas via two rather discrete pathways. These pathways are the dorsal longitudinal fasciculus of Schutz which terminates in the periventricular region of the hypothalamus, and the system of the mammillary peduncle which terminates largely in the mammillary body, but also projects to lateral regions of the hypothalamus, preoptic, and medial nucleus of the septal region. Thus, it appears possible that a medial region of the caudal midbrain, receiving an afferent supply from several

nonspecific (probably largely nociceptive and visceral) sensory systems, can exert a direct influence upon endocrine and autonomic mechanisms represented in the hypothalamus. Of further interest is Nauta's observation (143) that this mesencephalic region has extensive reciprocal connections with the limbic system and thus appears as the lower pole of a "limbic system—midbrain circuit" in which the hypothalamus seems to represent an intercalated mechanism. The functional significance of this cyclic apparatus awaits further study.

The role of the hypothalamus in the regulation of ACTH secretion has received continued attention. Hume (175) has reported that hypothalamic lesions which are most effective in blocking pituitary-adrenal cortical responses to stress in dogs are in the anteromedial region. Mason (176) observed marked plasma 17-OH-CS elevations following electrical stimulation continuing 90 min. through chronically implanted electrodes of the medial hypothalamus in the conscious monkey, thus confirming earlier work in which other indices of ACTH secretion were used. No change from normal 17-OH-CS patterns was observed during similar stimulations of the putamen. Fortier *et al.* (177) found that pituitary stalk section and prevention of regeneration of portal vessels reduced or abolished the lymphopenic response to restraint and cold exposure but exerted little effect on the response to laparotomy or the adrenal ascorbic acid depletion following unilateral adrenalectomy. This data is interpreted to indicate that some stimuli act through the brain and that some act directly through the blood stream to increase ACTH secretion. Poirier & Cordeau (178) have found different patterns of blood glucose, granulocyte, eosinophil, and lymphocyte responses to cold and immobilization in monkeys with lesions in three different hypothalamic areas.

Knigge & Bierman (179) present evidence of a central nervous system influence upon cold-induced acceleration of thyroidal I^{131} release in that slower responses than normal ensue in hamsters with hypothalamic lesions. On the basis that grafted ovarian tissue in the hypothalamus (paraventricular nucleus region) inhibited gonadotrophic activity in rats while similar transplants in the adenohypophysis did not, it has been suggested that estrogens affect gonadotrophin secretion exclusively through a nervous mechanism (180). Anand & Malkani (181) reported that stimulation of the medial part of the anterior and middle hypothalamic regions following the menstrual flow produced evidence of ovulation, while stimulation following ovulation produced menstrual flow in monkeys. In another study, Anand *et al.* (182) found prolonged diestrus and irregularities of vaginal epithelial cycles in rats with bilateral lesions in the medial part of the anterior and middle hypothalamic regions. The authors conclude that these hypothalamic regions are concerned with the regulation of gonadotrophin secretion. Bogdanove (183) presents evidence, based upon target organ studies and gonadotrophin assay of pituitary tissue in rats with various hypothalamic lesions, suggesting that specific and independent neural mechanisms regulate thyrotrophic and gonadotrophic secretions. In a study of hypothalamic influences on lactation, it was found that lesions in parts of the hypothalamus not in-

volving the pituitary stalk did not alter milk yield or litter growth in the rabbit (184). An extensive exploration of the relationships of the supraoptic and paraventricular nuclei to the regulation of secretion of the thyrotrophic, adrenocorticotrophic, gonadotrophic, and posterior pituitary hormones was reported by Olivecrona (185). He obtained evidence that these two nuclei and their fiber systems may be functionally different, the paraventricular nucleus being concerned with oxytocin secretion and the supraoptic nucleus having to do mainly with antidiuretic-vasopressor secretion.

French *et al.* (186) found a high incidence of gastrointestinal disease in monkeys subjected to repeated electrical stimulation of the hypothalamus and surrounding regions through chronically implanted electrodes for periods ranging from 30 to 86 days. Out of a group of nineteen animals selected as having received adequate stimulation, three showed focal lesions at the pyloric antrum, three had ulcers in the duodenum, and two had diffuse gastric changes. It appeared that a low midline axis in the hypothalamus was common to those animals with gastrointestinal disease. Sen & Anand (187) found marked increases in gastric secretory volume, acidity, and pepsin content as a result of electrical stimulation of preoptic, anteromedial hypothalamic, and anterior tegmental regions in conscious cats.

Vasoconstriction was found to be the most common response to electrical stimulation of various lower brain stem areas in bilaterally vagotomized cats and dogs under anesthesia (188). Sites were also observed in which stimulation produced increased force of myocardial contraction, unaffected by subsequent T_4 to T_8 transection of the cord. Sherrer & Friedman (189) observed two categories of systolic arterial pressure response in rats receiving prolonged electrical stimulation of the posterior hypothalamus: first, an initial pressor response of short latency and duration and, second, a sustained pressor response of long duration in initially hypotensive animals. The latter effect persists after bilateral adrenalectomy, vagotomy, or spinal cord transection but is abolished by lesions of the median eminence in the region of the tuber cinereum. Redgate & Gellhorn (190) concluded, on the basis of effects of acute hypothalamic lesions on rate and depth of respiration in anesthetized cats, that impulses from the lateral hypothalamus exert a tonic facilitating action on lower respiratory mechanisms. Cross & Glover (191) found that electrical stimulation of the dorsal, lateral, or posterior hypothalamus caused immediate contraction of the seminal vesicles, followed by a delayed contractile effect. The immediate response was abolished by section of the hypogastric nerves and the delayed effect by adrenalectomy.

Some aspects of the neural control of hunger, appetite, and satiety have been reviewed by Brobeck (192). In a series of studies in the rat, Morrison and co-workers have found transient effects of sham operations in the hypothalamus on food and water intake (193), adipsia and aphagia after lateral subthalamic lesions (194), and present evidence supporting the interesting possibility that the median forebrain bundle may be as important as the lateral area proper in the control of food and water intake (195). Kennedy (196) reports that the marked increase in food intake and development of

obesity occurring in adult rats, following lesions in the ventromedial hypothalamic nuclei, do not appear in weanling rats until the age when intake of food normally falls. Teitelbaum & Campbell (197) have studied the ingestion patterns in hyperphagic and normal rats on both liquid and solid diets. Andersson & Wyrwicka (198) report that when drinking was firmly established as a motor conditioned response in goats it was possible to elicit the same reaction by electrical stimulation of the anterior hypothalamus. Freedman & Fenichel (199) reported that bilaterally symmetrical lesions in the reticular formation at the level of the superior colliculus inhibited anaphylaxis in sensitized guinea pigs, and the possibility that heightened readiness for response of midbrain mechanisms may be a factor in some aspects of allergy is discussed. Birzis & Hemingway (200) described 21 sites, which were within the lesion-determined "shivering pathway" in the cat's brain stem, where electrical stimulation causes a tremor like natural shivering. In the hypothalamus this effect was obtained in the medial part of the tuberal region between the mammillothalamic tract and the fornix.

PHARMACOLOGICAL STUDIES AND REGULATION OF VISCERAL FUNCTIONS

Of the vast neuropharmacological literature, only a small, selected part considered particularly pertinent to the central regulation of visceral functions will be covered here. The central-acting agent may be regarded, along with ablation, electrical stimulation and recording, and neurochemical techniques, as one of the few available tools for studying localization of functional mechanisms within the nervous system. As evidence grows that drugs can affect selective aspects of psychological, particularly emotional, functions and as convergence of information occurs from the use of drugs together with neurochemical, electrophysiological, and other measurements, it appears hopeful that pharmacological tools may add increasingly to our understanding of the functional organization of the nervous system.

The recent development of many drugs, particularly the tranquilizers, which have selective effects on emotional processes has stimulated interest in the investigation of the changes in visceral function accompanying drug-altered emotional states. For example, many experimental studies have shown emotional arousal to be associated with increased adrenal activity. One might expect, then, that reserpine, for example, which reduces emotional reactivity would have a corresponding effect on adrenal function. Some of the experiments directed at this problem have yielded seemingly paradoxical results. Muscholl & Vogt (201) observed significant elevations of plasma epinephrine, but not of norepinephrine, in rabbits between 35 and 90 min. after receiving 1 to 2.3 mg. per kg. of reserpine daily. The same authors (202) reported that small daily doses of reserpine caused marked depletion of catecholamine concentrations throughout the peripheral sympathetic system. Egdaahl *et al.* (203) observed elevations of 17-OH-CS in adrenal vein blood of dogs shortly after reserpine administration. Harwood & Mason (204) found marked elevations in peripheral plasma 17-OH-CS levels in monkeys with single intravenous injections of reserpine in doses from 0.05 to 1.0 mg. per kg. Such findings would indicate stimulation of pituitary-

adrenal cortical and sympathetic-adrenal medullary activity with acute administration of reserpine. Monkeys studied 22 hr. following daily doses of reserpine, however, had normal plasma 17-OH-CS levels and failed to show the usual corticosteroid and behavioral responses to a conditioned emotional stimulus (16). When reserpine administration was discontinued after two weeks of daily 0.75 mg./kg. doses, it took about three weeks for normal plasma 17-OH-CS responses to occur following the conditioned emotional stimulus. It would appear then that one must distinguish between transient responses immediately following reserpine administration and those which persist for much longer periods, particularly with chronic administration of the drug. This is also supported by the findings of Domino & Rech (205) who observed that biphasic arterial pressure effects of this sort may be demonstrated in unanesthetized dogs following reserpine administration. An initial hypertensive effect, usually maximal in approximately 20 min., may precede the well-known hypotensive effect. A diphasic EEG response was also observed in these animals (205).

In studies of other visceral responses, Barraclough & Sawyer (206) found that reserpine and chlorpromazine blocked ovulation in mature, cyclic rats when injected prior to 2 p.m. on the day of proestrus. Reserpine in graded doses above 0.25 mg. per kg. had increasing effects on gastric secretion, as measured by total volume and free hydrochloric acid, in human subjects. The stimulating effect of reserpine on gastric secretion could be blocked by large doses of an anticholinergic drug, scopolamine methylbromide (Pamine), but not by vagotomy or sympathectomy (207).

On the basis of various autonomic measurements in humans, DiMascio *et al.* (208) conclude that lysergic acid diethylamide produced general sympathetic excitation, reaching a peak in about 3 to 4 hr. Manger *et al.* (209) observed elevations in mean levels of plasma epinephrinelike substance 1/2 and 2 hr. after 400 mg. of mescaline were given orally to normal human subjects, but this effect was not observed in four psychotic patients.

The discovery of depletion of brain serotonin or 5-hydroxytyramine (5-HT) levels following reserpine administration (210, 211) has helped to renew enthusiasm for neurochemical research. The extensive investigative effort concentrated on serotonin during the past four years has been reviewed by Page (212), who included discussion of brain serotonin and its significance. MacLean *et al.* (213) found that reserpine administration produced distinctive changes in bioelectrical activity of the hippocampus and posterior hypothalamus and subsequently noticed that the time pattern for the depletion and restoration of brain 5-HT levels after reserpine administration, reported by Pletscher *et al.* (210), was strikingly similar to the course of the electroencephalographic changes following reserpine. This observation prompted a study (214) of 5-HT content of limbic system structures in the dog brain, in which the pyriform cortex, hippocampus, amygdala, septal region, hypothalamus, and caudate nucleus were all found to have 5-HT levels of over 200 μ g. per gm. while four neocortical areas studied ranged from 13 to 85 μ g. per gm. The functional implications of these interesting findings await physiological investigation but caution in this area is

indicated on several counts. It has been shown recently that norepinephrine (215) and 3-hydroxytyramine (216) also disappear from brain tissue rapidly following reserpine administration. In addition, another group of drugs which are benzoquinolizine derivatives has been found to cause 5-HT release from the brain, and it is not yet reported how closely the behavioral effects of these agents resemble those of reserpine (217). Although many separate findings point to an important neurophysiological role for 5-HT, it seems clear that more direct evidence on the mechanism of its action on brain cells and evidence relating 5-HT changes to specific neural or psychological stimuli in the intact animal would be valuable in elucidating such a role. For broad coverage of other recent neuropharmacological research, reviews by Wikler (218) and Bradley & Elkes (219) are recommended.

In conclusion, the author wishes to acknowledge regretfully that it has been necessary to omit mention of much pertinent and fundamental research of the past year in order to achieve even limited coverage of the central aspects of visceral regulation. It is hoped that at least some of the omitted studies dealing with visceral receptors, peripheral autonomic nerves, humoral and pharmacological aspects of neuronal function, and related subjects may be covered in the succeeding volume.

LITERATURE CITED

1. Mason, J. W., Harwood, C. T., and Rosenthal, N., *Am. J. Physiol.*, **190**, 429-33 (1957)
2. Mason, J. W., Brady, J. V., and Sidman, M., *Endocrinology*, **60**, 741-52 (1957)
3. Price, D. B., Thaler, M., and Mason, J. W., *Arch. Neurol. Psychiat.*, **77**, 646-56 (1957)
4. Sabshin, M., Hamburg, D. A., Grinker, R. R., Persky, H., Basowitz, H., Korchin, S. J., and Chevalier, J. A., *Arch. Neurol. Psychiat.*, **78**, 207-19 (1957)
5. Holcombe, R. B., *Acta Endocrinol.*, Suppl. 34, 1-100 (1957)
6. Sloane, R. B., Saffran, M., and Cleghorn, R. A., *Arch. Neurol. Psychiat.*, **79**, 549-53 (1958)
7. Persky, H., Grinker, R. R., Hamburg, D. A., Sabshin, M. A., Korchin, S. J., Basowitz, H., and Chevalier, J. A., *Arch. Neurol. Psychiat.*, **76**, 549-58 (1956)
8. Estes, W. K., and Skinner, B. F., *J. Exptl. Psychol.*, **29**, 390-400 (1941)
9. Brady, J. V., and Hunt, H. F., *J. Psychol.*, **40**, 313-24 (1955)
10. Sidman, M., *Science*, **118**, 157-58 (1953)
11. Dinsmoor, J. A., *Quart. J. Exptl. Psychol.*, **4**, 27-45 (1952)
12. Hunt, H. F., and Brady, J. V., *J. Comp. and Physiol. Psychol.*, **48**, 305-10 (1955)
13. Mason, J. W., Mangan, G. F., Conrad, D. G., Cain, C., and Brady, J. V., *Program Endocrine Soc. Meeting*, 20 (1957)
14. Solomon, R., and Wynne, L. C., *Am. Psychologist*, **5**, 264 (1950)
15. Mirsky, I. A., Miller, R., and Stein, M., *Psychosomat. Med.*, **15**, 574-88 (1953)
16. Mason, J. W., and Brady, J. V., *Science*, **124**, 983-84 (1956)
17. Mason, J. W., *J. Appl. Physiol.*, **12**, 130-33 (1958)
18. Porter, R. W., Brady, J. V., Conrad, D. G., Mason, J. W., Galambos, R., and Rioch, D. McK., *Psychosomat. Med.* (In press)
19. Brady, J. V., Porter, R. W., Conrad, D. G., and Mason, J. W., *J. Exptl. Analysis of Behavior*, **1**, 69-72 (1958)

20. Habermann, R. T., and Williams, F. P., *Am. J. Vet. Research*, **18**, 419-26 (1957)
21. Weisz, J. D., *Psychosomat. Med.*, **19**, 61-73 (1957)
22. Sawrey, W. L., Conger, J. J., and Turrell, E. S., *J. Comp. and Physiol. Psychol.*, **49**, 457-61 (1956)
23. Engel, G. L., Reichsman, F., and Segal, H. L., *Psychosomat. Med.*, **18**, 374-98 (1956)
24. Hamburg, D. A., Sabshin, M. A., Board, F. A., Grinker, R. R., Korchin, S. J., Basowitz, H., Heath, H., and Persky, H., *Arch. Neurol. Psychiat.*, **79**, 415-26 (1958)
25. Persky, H., Hamburg, D. A., Basowitz, H., Grinker, R. R., Sabshin, M., Korchin, S. J., Herz, M., Board, F. A., and Heath, H. A., *Arch. Neurol. Psychiat.*, **79**, 434-47 (1958)
26. Board, F., Persky, H., and Hamburg, D. A., *Psychosomat. Med.*, **18**, 324-33 (1956)
27. Board, F., Wadeson, R., and Persky, H., *Arch. Neurol. Psychiat.*, **78**, 612-20 (1957)
28. Persky, H., *Arch. Neurol. Psychiat.*, **78**, 95-100 (1957)
29. Persky, H., *J. Clin. Endocrinol. and Metabolism*, **17**, 760-65 (1957)
30. Glickstein, M., Chevalier, J. A., Korchin, S. J., Basowitz, H., Sabshin, M., Hamburg, D. A., and Grinker, R. R., *Arch. Neurol. Psychiat.*, **78**, 101-6 (1957)
31. Thaler, M., Weiner, H., and Reiser, M. F., *Psychosomat. Med.*, **19**, 228-39 (1957)
32. Gottschalk, L. A., and Kaplan, S., *Arch. Neurol. Psychiat.*, **79**, 688-96 (1958)
33. Romanoff, L. P., Rodriguez, R. M., Seelye, J. M., and Pincus, G., *J. Clin. Endocrinol. and Metabolism*, **17**, 777-85 (1957)
34. Stevenson, J. A. F., Derrick, J. B., Hobbs, G. E., and Metcalf, E. V., *Arch. Neurol. Psychiat.*, **78**, 312-20 (1957)
35. Frohman, C. E., Goodman, M., Luby, E. D., Beckett, P. G. S., and Senf, R., *Arch. Neurol. Psychiat.*, **79**, 730-34 (1958)
36. Ostfeld, A. M., Abood, L. G., and Marcus, D. A., *Arch. Neurol. Psychiat.*, **79**, 317-22 (1958)
37. Abood, L. G., Gibbs, F. A., and Gibbs, E., *Arch. Neurol. Psychiat.*, **77**, 643-45 (1957)
38. Horwitt, M. K., Meyer, B. J., Meyer, A. C., Harvey, C. C., and Haffron, D., *Arch. Neurol. Psychiat.*, **78**, 275-82 (1957)
39. Hill, S. R., *et al.*, *Arch. Internal Med.*, **97**, 269-98 (1956)
40. Bliss, E. L., Migeon, C. J., Branch, C. H. H., and Samuels, L. T., *Psychosomat. Med.*, **18**, 56-76 (1956)
41. Connell, A. M., Cooper, J., and Redfearn, J. W., *Acta Endocrinol.*, **27**, 179-94 (1958)
42. Venning, E. H., Dyrenfurth, I., and Beck, J. C., *J. Clin. Endocrinol. and Metabolism*, **17**, 1005-7 (1957)
43. Jones, G. M., *Quart. J. Exptl. Physiol.*, **42**, 390-97 (1957)
44. Yessler, P. G., Reiser, M. F., and Rioch, D. McK., *J. Am. Med. Assoc.* (In press)
45. Weiner, H., Thaler, M., Reiser, F., and Mirsky, I., *Psychosomat. Med.*, **19**, 1-10 (1957)
46. Helmreich, M. L., Jenkins, D., and Swan, H., *Surgery*, **41**, 895-909 (1957)
47. Viikari, S. J., and Thomasson, B. H., *Acta Endocrinol.*, **24**, 361-69 (1957)
48. Weichselbaum, T. E., Elman, R., and Margraf, H. W., *J. Clin. Endocrinol. and Metabolism*, **17**, 1158-67 (1957)

49. Moore, F. D., Steenberg, R. W., Ball, M. R., Wilson, G. M., and Myrden, J. A., *Ann. Surgery*, **141**, 145-74 (1955)
50. Reece, M. W., Edwards, K. M., and Jepson, R. P., *Surgery*, **42**, 669-80 (1957)
51. Edwards, K. M., Jepson, R. P., and Reece, M. W., *J. Clin. Endocrinol. and Metabolism*, **17**, 1460-65 (1957)
52. Hale, H. B., Sayers, G., Sydnor, K. L., Sweat, M. L., and Van Fossan, D. D., *J. Clin. Invest.*, **36**, 1642-46 (1957)
53. MacFarlane, W. V., and Robinson, K. W., *J. Appl. Physiol.*, **11**, 199-200 (1957)
54. Appleby, J. I., and Norymberski, J. K., *J. Endocrinol.*, **15**, 310-19 (1957)
55. Little, B., Vance, V. K., and Rossi, E., *J. Clin. Endocrinol. and Metabolism*, **18**, 49-53 (1958)
56. Birke, G., Gemzell, C. A., Plantin, L. O., and Robbe, H., *Acta Endocrinol.*, **27**, 389-402 (1958)
57. Wexler, B. C., Dolgin, A. E., and Tryczynski, E. W., *Endocrinology*, **61**, 488-99 (1957)
58. Marks, L. J., Weiss, D. M., Leftin, J. H., and Rossmeisl, E. C., *J. Clin. Endocrinol. and Metabolism*, **18**, 235-45 (1958)
59. Jakobson, T., *Acta Endocrinol.*, **27**, 432 (1958)
60. Clayton, G. W., Bell, W. R., and Guillemin, R., *Proc. Soc. Exptl. Biol. Med.*, **96**, 777-79 (1957)
61. McCann, S. M., *Endocrinology*, **60**, 664-76 (1957)
62. de Wied, D., *Acta Endocrinol.*, **24**, 200-8 (1957)
63. McDonald, R. K., Wagner, H. N., and Weise, V. K., *Proc. Soc. Exptl. Biol. Med.*, **96**, 652-55 (1957)
64. Shapiro, S., Marmorston, J., and Sobel, H., *Endocrinology*, **62**, 278-82 (1958)
65. Shapiro, S., Marmorston, J., and Sobel, H., *Am. J. Physiol.*, **192**, 58-62 (1958)
66. Long, C. N. H., and Bonnycastle, M. F. M., *Can. J. Biochem. and Physiol.*, **35**, 929-33 (1957)
67. Ely, R. S., Hughes, E. R., and Kelley, V. C., *J. Clin. Endocrinol. and Metabolism*, **18**, 190-207 (1958)
68. Weichselbaum, T. E., and Margraf, H. W., *J. Clin. Endocrinol. and Metabolism*, **17**, 959-65 (1957)
69. Wilson, H., Borris, J. J., and Garrison, M. M., *J. Clin. Endocrinol. and Metabolism*, **18**, 643-61 (1958)
70. Rauschkolb, E. W., and Farrell, G. L., *Endocrinology*, **59**, 526-31 (1956)
71. Rauschkolb, E. W., and Farrell, G. L., *J. Clin. Endocrinol. and Metabolism*, **16**, 915-16 (1956)
72. Farrell, G. L., Rauschkolb, E. W., Fleming, R. B., and Yatsu, F. M., *Program Endocrine Soc. Meeting*, 32 (1957)
73. Davis, J. O., Bahn, R. C., Goodkind, M. J., and Ball, W. C., *Am. J. Physiol.*, **191**, 329-38 (1957)
74. Casey, J. H., Bickel, E. Y., and Zimmerman, B., *Surg., Gynecol. Obstet.*, **105**, 179-85 (1957)
75. Driscoll, T. E., *Am. J. Physiol.*, **191**, 140-44 (1957)
76. Ball, W. C., Jr., and Davis, J. O., *Am. J. Physiol.*, **191**, 339-41 (1957)
77. Ball, W. C., Jr., Davis, J. O., Pechet, M. M., and Goodkind, M. J., *Am. J. Physiol.*, **187**, 584 (1956)
78. Davis, J. O., Goodkind, M. J., Pechet, M. M., and Ball, W. C., Jr., *Am. J. Physiol.*, **187**, 45-50 (1956)
79. Bartter, F. C., Liddle, G. W., Duncan, L. E., Jr., Barber, J. K., and Delea, C., *J. Clin. Invest.*, **35**, 1306-15 (1956)

80. Orti, E., Ralli, E. P., Laken, B., and Dumm, M. E., *Am. J. Physiol.*, **191**, 323-28 (1957)
81. Laidlaw, J. C., Cohen, M., and Gornall, A. G., *J. Clin. Endocrinol. and Metabolism*, **18**, 222-24 (1958)
82. Baulieu, E. E., de Vigan, M., Bricaire, H., and Jayle, M. F., *J. Clin. Endocrinol. and Metabolism*, **17**, 1478-82 (1957)
83. Nowaczynski, W., Koiv, E., and Genest, J., *Can. J. Biochem. and Physiol.*, **35**, 425-43 (1957)
84. Moolenaar, A. J., *Acta Endocrinol.*, **25**, 161-72 (1957)
85. Halme, A., Pekkarinen, A., and Turunen, M., *Acta Endocrinol.*, Suppl. 32, 1-52 (1957)
86. Watts, D. T., and Bragg, A. D., *Proc. Soc. Exptl. Biol. Med.*, **96**, 609-12 (1957)
87. Manger, W. M., Bollman, J. L., Maher, F. T., and Berkson, J., *Am. J. Physiol.*, **190**, 310-16 (1957)
88. Griswold, R. L., *J. Appl. Physiol.*, **12**, 117-20 (1958)
89. Gray, I., and Beetham, W. P., *Proc. Soc. Exptl. Biol. Med.*, **96**, 636-38 (1957)
90. Goldfien, A., Zileli, M. S., Despointes, R. H., and Bethune, J. E., *Endocrinology*, **62**, 749-57 (1958)
91. Weil-Malherbe, H., and Bone, A. D., *Biochem. J.*, **67**, 65-72 (1957)
92. Jones, R. T., and Blake, W. D., *J. Appl. Physiol.*, **12**, 448-52 (1958)
93. Goldfien, A., and Karler, R., *Science*, **127**, 1292-93 (1958)
94. Young, J. G., and Fischer, R. L., *Science*, **127**, 1390 (1958)
95. Mangan, G. F., Jr., and Mason, J. W., *J. Lab. Clin. Med.*, **51**, 484-93 (1958)
96. Schwartz, A. E., and Roberts, K. E., *Surgery*, **42**, 814-18 (1957)
97. MacLagan, N. F., Bowden, C. H., and Wilkinson, J. H., *Biochem. J.*, **67**, 5-11 (1957)
98. Pind, K., *Acta Endocrinol.*, **26**, 263-72 (1957)
99. Thorn, N. A., *Physiol. Revs.*, **38**, 169-95 (1958)
100. Theobald, G. W., *J. Physiol. (London)*, **141**, 30P (1958)
101. Buchborn, E., *Endocrinology*, **61**, 375-79 (1957)
102. Taylor, N. B. G., Hunter, J., and Johnson, W. H., *Can. J. Biochem. and Physiol.*, **35**, 1017-27 (1957)
103. Migeon, C. J., Keller, A. R., Lawrence, B., and Shepard, T. H., *J. Clin. Endocrinol. Metabolism*, **17**, 1051-62 (1957)
104. Brinck-Johnsen, T., and Eik-Nes, K., *Endocrinology*, **61**, 676-83 (1957)
105. Brown, J. B., Bulbrook, R. D., and Greenwood, F. C., *J. Endocrinol.*, **16**, 41-48 (1957)
106. Brown, J. B., Bulbrook, R. D., and Greenwood, F. C., *J. Endocrinol.*, **16**, 49-56 (1957)
107. Bulbrook, R. D., Greenwood, F. C., and Williams, P. C., *J. Endocrinol.*, **15**, 206-10 (1957)
108. Smith, O. W., and Blackham, N. N., *Acta Endocrinol.*, **25**, 133-60 (1957)
109. West, C. D., Damast, B., and Pearson, O. H., *J. Clin. Endocrinol. and Metabolism*, **18**, 15-27 (1958)
110. Cole, H. H., Hamburger, C., and Neimann-Sorensen, A., *Acta Endocrinol.*, **26**, 286-98 (1957)
111. Henry, S. S., and Van Dyke, H. B., *J. Endocrinol.*, **16**, 310-25 (1958)
112. Skom, J. H., and Talmage, D. W., *J. Clin. Invest.*, **37**, 783-86 (1958)
113. Clemmedson, C. J., *Am. J. Physiol.*, **190**, 467-72 (1957)
114. Baugh, C. W., Bird, G. S., Brown, G. M., Lennox, C. S., and Semple, R. E., *J. Physiol. (London)*, **140**, 347-58 (1958)
115. Le Blanc, J. A., and Rosenberg, F. J., *J. Appl. Physiol.*, **11**, 344-48 (1957)

116. Hix, E. L., *Am. J. Physiol.*, **192**, 191-97 (1958)
117. Berman, L. B., and Rose, J. C., *Am. J. Physiol.*, **193**, 260-62 (1958)
118. Abrahams, V. C., and Pickford, M., *J. Physiol. (London)*, **141**, 527-34 (1958)
119. Wagner, H. N., Jr., *J. Clin. Invest.*, **36**, 1319-27 (1957)
120. Smith, C. C., and Ansevin, A., *Proc. Soc. Exptl. Biol. Med.*, **96**, 428-32 (1957)
121. Smith, C. C., and Ansevin, A., *Proc. Soc. Exptl. Biol. Med.*, **96**, 432-37 (1957)
122. Davis, R. C., *J. Comp. and Physiol. Psychol.*, **50**, 524-29 (1957)
123. Lewis, D. H., Deitz, G. W., Wallace, J. D., and Brown, J. R., *Circulation*, **16**, 764-75 (1957)
124. Huff, R. L., Parrish, D., and Crockett, W., *Circulation Research*, **5**, 395-400 (1957)
125. Hirschowitz, B. I., *Physiol. Revs.*, **37**, 475-511 (1957)
126. Soiva, K., Castren, O., and Koskinen, P., *Acta Endocrinol.*, **27**, 123-28 (1958)
127. Thomas, J. E., *Physiol. Revs.*, **37**, 453-74 (1957)
128. Farrar, J. T., and Bernstein, J. S., *Program World Congress Gastroenterol.*, 237-38 (1958)
129. Davis, R. C., Garafolo, L., and Gault, F. P., *J. Comp. and Physiol. Psychol.*, **50**, 519-23 (1957)
130. Texter, E. C., Jr., Smith, H. W., Moeller, H. C., and Barborka, C. J., *Gastroenterology*, **32**, 1013-24 (1957)
131. Smith, H. W., Texter, E. C., Jr., Stickley, J. H., and Barborka, C. J., *Gastroenterology*, **32**, 1025-47 (1957)
132. Morrison, S. D., Lin, H. J., Eckel, H. E., Van Itallie, T. B., and Mayer, J., *Am. J. Physiol.*, **193**, 4-8 (1958)
133. Lane, A., Ivy, A. C., and Ivy, E. K., *Am. J. Physiol.*, **192**, 221-28 (1957)
134. Lin, T. M., and Alphin, R. S., *Am. J. Physiol.*, **192**, 23-26 (1958)
135. Smith, M., and Duffy, M., *J. Comp. and Physiol. Psychol.*, **50**, 601-8 (1957)
136. Weiss, B., *Science*, **127**, 467-68 (1958)
137. Harker, J. E., *Biol. Revs., Cambridge Phil. Soc.*, **33**, 1-52 (1958)
138. Lewis, P. R., and Lobban, M. C., *Quart. J. Exptl. Physiol.*, **42**, 356-71 (1957)
139. Lewis, P. R., and Lobban, M. C., *Quart. J. Exptl. Physiol.*, **42**, 371-86 (1957)
140. Halberg, F., French, L. A., and Gully, R. J., *J. Appl. Physiol.*, **12**, 381-84 (1958)
141. Ferguson, D. J., Visscher, M. B., Halberg, F., and Levy, L. M., *Am. J. Physiol.*, **190**, 235-38 (1957)
142. Iampietro, P. F., Buskirk, E. R., Bass, D. E., and Welch, B. E., *J. Appl. Physiol.*, **11**, 349-52 (1957)
143. Nauta, W. J. H., *Brain*, **81**, 319-40 (1958)
144. Gloor, P., *Hypothalamic-Hypophyseal Interrelationships*, 74-113 (Charles C Thomas, Publisher, Springfield, Ill., 156 pp., 1956)
145. Brady, J. V., *Interdisciplinary Research in the Behavioral, Biological, and Biochemical Sciences* (Univ. of Wisconsin Press, Madison, Wis., in press)
146. MacLean, P. D., *Electroencephal. and Clin. Neurophysiol.*, **4**, 407-18 (1956)
147. MacLean, P. D., *Ann. Rev. Physiol.*, **19**, 397-416 (1957)
148. Schreiner, L., and Kling, A., *J. Neurophysiol.*, **16**, 643-59 (1953)
149. Wood, C. D., *Neurology*, **8**, 215-20 (1958)
150. Kling, A., and Hutt, P. J., *Arch. Neurol. Psychiat.*, **79**, 511-17 (1958)
151. De Molina, A. F., and Hunsperger, R. W., *J. Physiol. (London)*, **138**, 29-30 (1957)
152. Shealy, C. N., and Peele, T. L., *J. Neurophysiol.*, **20**, 125-39 (1957)
153. Wood, C. D., Schottelius, B., Frost, L. L., and Baldwin, M., *Neurology*, **8**, 477-80 (1958)

154. Mason, J. W., *Intern. Symposium on Reticular Formation of Brain* 645-62 (Little, Brown & Co., Boston, Mass., 766 pp., 1958)
155. Bunn, J. P., and Everett, J. W., *Proc. Soc. Exptl. Biol. Med.*, **96**, 369-71 (1957)
156. Sen, R. N., and Anand, B. K., *Indian J. Med. Research*, **45**, 515-21 (1957)
157. Andersson, B., *Acta Physiol. Scand.*, **41**, 90-100 (1957)
158. Andersson, B., and Persson, N., *Acta Physiol. Scand.*, **41**, 277-82 (1957)
159. Bond, D. D., Randt, C. T., Bidder, G. T., and Rowland, V., *Arch. Neurol. Psychiat.*, **78**, 143-62 (1957)
160. MacLean, P. D., *Arch. Neurol. Psychiat.*, **78**, 113-27 (1957)
161. MacLean, P. D., *Arch. Neurol. Psychiat.*, **78**, 128-42 (1957)
162. MacLean, P. D., *Yale J. Biol. and Med.*, **28**, 380-95 (1955-56)
163. Thomas, G. J., and Otis, L. S., *J. Comp. and Physiol. Psychol.*, **51**, 161-66 (1958)
164. Weiskrantz, L., and Wilson, W. A., Jr., *J. Comp. and Physiol. Psychol.*, **51**, 167-71 (1958)
165. Mirsky, A. F., Rosvold, H. E., and Pribram, K. H., *J. Neurophysiol.*, **20**, 588-601 (1957)
166. Alonso-de-Florida, F., and Delgado, J. M. R., *Am. J. Physiol.*, **193**, 223-29 (1958)
167. Penfield, W., and Milner, B., *Arch. Neurol. Psychiat.*, **79**, 475-97 (1958)
168. Walker, A. E., *Arch. Neurol. Psychiat.*, **78**, 543-52 (1957)
169. Gloor, P., *Arch. Neurol. Psychiat.*, **77**, 247-58 (1957)
170. Powell, E. W., Haggart, J., Goodfellow, E., and Niemer, W. T., *Neurology*, **7**, 689-96 (1957)
171. Adey, W. R., Sunderland, S., and Dunlop, C. W., *Electroencephal. and Clin. Neurophysiol.*, **9**, 309-24 (1957)
172. Davis, G. D., *Neurology*, **8**, 135-39 (1958)
173. Rosvold, H. E., and Delgado, J. M. R., *J. Comp. and Physiol. Psychol.*, **49**, 365-72 (1956)
174. Nauta, W. J. H., and Kuypers, H. G. J. M., *Intern. Symposium on Reticular Formation of Brain*, 3-30 (Little, Brown & Co., Boston, Mass., 766 pp., 1958)
175. Hume, D. M., *Intern. Symposium on Reticular Formation of Brain*, 231-48 (Little, Brown & Co., Boston, Mass., 766 pp., 1958)
176. Mason, J. W., *Am. J. Physiol.* (In press)
177. Fortier, C., Harris, G. W., and McDonald, I. R., *J. Physiol. (London)*, **136**, 344-63 (1957)
178. Poirier, L. J., and Cordeau, J. P., *Am. J. Physiol.*, **191**, 148-52 (1957)
179. Knigge, K. M., and Bierman, S. M., *Am. J. Physiol.*, **192**, 625-30 (1958)
180. Flerko, B., and Szentagothai, J., *Acta Endocrinol.*, **26**, 121-27 (1957)
181. Anand, B. K., and Malkani, P. K., *Indian J. Med. Research*, **45**, 499-502 (1957)
182. Anand, B. K., Malkani, P. K., and Sikand, S., *Indian J. Med. Research*, **45**, 503-6 (1957)
183. Bogdanove, E. M., *Endocrinology*, **60**, 689-97 (1957)
184. Donovan, B. T., and Van der Werff ten Bosch, J. J., *J. Physiol. (London)*, **137**, 410-20 (1957)
185. Olivecrona, H., *Acta Physiol. Scand.*, **40**, Suppl. 136, 1-178 (1957)
186. French, J. D., Porter, R. W., Cavanaugh, E. B., and Longmire, R. L., *Psychosomat. Med.*, **19**, 209-20 (1957)
187. Sen, R. N., and Anand, B. K., *Indian J. Med. Research*, **45**, 507-13 (1957)
188. Peiss, C. N., *J. Physiol. (London)*, **141**, 500-9 (1958)
189. Sherrer, H. F., and Friedman, S. M., *Acta Endocrinol.*, **27**, 89-98 (1958)
190. Redgate, E. S., and Gellhorn, E., *Am. J. Physiol.*, **193**, 189-94 (1958)

191. Cross, B. A., and Glover, T. D., *J. Endocrinol.*, **16**, 385-95 (1958)
192. Brobeck, J. R., *Yale J. Biol. and Med.*, **29**, 565-74 (1957)
193. Morrison, S. D., and Mayer, J., *Am. J. Physiol.*, **191**, 255-58 (1957)
194. Morrison, S. D., and Mayer, J., *Am. J. Physiol.*, **191**, 248-54 (1957)
195. Morrison, S. D., Barnett, R. J., and Mayer, J., *Am. J. Physiol.*, **193**, 230-34 (1958)
196. Kennedy, G. C., *J. Endocrinol.*, **16**, 9-17 (1957)
197. Teitelbaum, P., and Campbell, B. A., *J. Comp. and Physiol. Psychol.*, **51**, 135-41 (1958)
198. Andersson, B., and Wyrwicka, W., *Acta Physiol. Scand.*, **41**, 194-98 (1957)
199. Freedman, D. X., and Fenichel, G., *Arch. Neurol. Psychiat.*, **79**, 164-69 (1958)
200. Birzis, L., and Hemingway, A., *J. Neurophysiol.*, **20**, 91-99 (1957)
201. Muscholl, E., and Vogt, M., *Brit. J. Pharmacol.*, **12**, 532-35 (1957)
202. Muscholl, E., and Vogt, M., *J. Physiol. (London)*, **141**, 132-55 (1958)
203. Egdahl, R. H., Richards, J. B., and Hume, D. M., *Science*, **123**, 418 (1956)
204. Harwood, C. T., and Mason, J. W., *Endocrinology*, **60**, 239-46 (1957)
205. Domino, E. F., and Rech, R., *J. Pharmacol. Exptl. Therap.*, **121**, 171-82 (1957)
206. Barraclough, C. A., and Sawyer, C. H., *Endocrinology*, **61**, 341-51 (1957)
207. Rider, J. A., Moeller, H. C., and Gibbs, J. O., *Gastroenterology*, **33**, 737-44 (1957)
208. DiMascio, A., Greenblatt, M., and Hyde, R. W., *Am. J. Psychiat.*, **114**, 309-17 (1957)
209. Manger, W. M., Schwarz, B. E., Baars, C. W., Wakim, K. G., Bollman, J. T., Peterson, M. C., and Berkson, J., *Arch. Neurol. Psychiat.*, **78**, 396-412 (1957)
210. Pletscher, A., Shore, P. A., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, **116**, 84-89 (1956)
211. Shore, P. A., Pletscher, A., Tomich, E. G., Carlsson, A., Kuntzman, R., and Brodie, B. B., *Ann. N. Y. Acad. Sci.*, **66**, 609-17 (1957)
212. Page, I. H., *Physiol. Revs.*, **38**, 277-335 (1958)
213. MacLean, P. D., Flanigan, S., Flynn, J. P., Kim, C., and Stevens, J. R., *Yale J. Biol. and Med.*, **28**, 380-95 (1955-56)
214. Paasonen, M. K., MacLean, P. D., and Giarman, N. J., *J. Neurochem.*, **1**, 326-33 (1957)
215. Brodie, B. B., Olin, J. S., Kuntzman, R. G., and Shore, P. A., *Science*, **125**, 1293-94 (1957)
216. Carlsson, A., Lindqvist, M., Magnusson, T., and Waldeck, B., *Science*, **127**, 471 (1958)
217. Pletscher, A., *Science*, **126**, 507 (1957)
218. Wikler, A., *The Relation of Psychiatry to Pharmacology* (Williams and Wilkins Co., Baltimore, Md., 322 pp., 1957)
219. Bradley, P. B., and Elkes, J., *Brain*, **80**, 77-117 (1957)

HIGH FUNCTIONS OF THE NERVOUS SYSTEM^{1,2}

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A naive view of the brain that features anatomical laminae differentiated to sensory and motor functions, and still other laminae involved in pleasure and pain, learning, alerting or integrating, and other functions has gained ground at the expense of more complex theories. This trend back to less complex notions owes most perhaps to new and successful explorations for "higher" functions in "lower" centers of the brain. No one glibly downgrades the neocortex, but there seems to be a growing tendency to regard it as an amplifying and encoding apparatus which simplifies patterns and then feeds them back to the older, but perhaps "higher" integrating apparatuses of the midbrain, the diencephalon, and the paleocortex. No longer can the possibility be overlooked that the concept of "equipotentiality", which has enjoyed such a vogue, owed much to the fact that functions were sought in the neocortex which actually resided in subcortical or paleocortical structures. This is not to gainsay the thesis that experimental manipulations of the brain reveal both "specific" (localizable) and "general" (nonfocal)" effects (1, 2), but it is to say that work on the cortex in rats tended to cause an overemphasis on the general ones.

FUNCTIONAL DIFFERENTIATION

A set of concepts concerning the division of labor between the various systems of the brain, which guides current research, has grown out of an expanding set of researches including electronic recording, stimulation, and ablation of discrete points and structures in the relatively intact brains of live animals. There are three alternative, but converging, functional analyses of the brain that are importantly related to the data produced this year.

From the laboratory of the Walter Reed Army Institute of Research comes the set of concepts expressed by Galambos (3), which divides the brain into four main systems: (a) classical afferent pathways passing through the thalamus and forming one system with the specific areas of the cortex; (b) the more recently discovered efferent sensory systems which arise in the cortex and make their way right back down the classical pathways making synaptic relations with ascending fibers at every level in the classical system; (c) the reticular system receiving collaterals from the classical pathways and projecting these by a different route to the cortex, also receiving offshoots from efferent sensory systems and participating in the control of lower cen-

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ters; and (d) the limbic midbrain circuit—fibers starting in the midbrain, making their way to the hippocampus, amygdala, hypothalamus, and septal area, and then returning to the reticular formation.

Functionally, Galambos supposes that the classical pathways and the efferent sensory systems acting in consort have to do not only with conveying informational patterns from the periphery but also provide places to search for the lasting connections involved in learning and memory. The reticular formation is presumed to be involved in alerting and attention, and the limbic system is thought to handle more-or-less rigid approach and avoidance patterns involved in the maintenance of the organism—survival and well-being. In summary this view suggests that learning mechanisms might be in the afferent and efferent primary systems, arousal mechanisms in the reticular system, and motivational mechanisms in the limbic system.

An alternative view expressed by Pribram (4, 5) starts out by grouping, on the one hand, the reticular and limbic parts of the brain as the internal systems and, on the other, the sensory apparatuses as the external systems. This distinction is made on an anatomical and functional basis. The external systems are said to receive a sensory, mode-specific input through tracts made up of large, long nerve fibers. These tracts are so constituted as to maintain a topological correspondence between the organization of receptor events and those occurring in the forebrain. The external systems function in discrimination tasks.

The internal systems are not similarly involved in momentary patterns (spatial arrays) but have a function which involves maintenance of tone through an activation pool, or modulation of excitability on the basis of sequential or cyclic patterns. Pribram states that the internal core of the forebrain receives a nonspecific input through systems made up of fine, short nerve fibers diffusely connected by many synapses. These systems are so constituted as to influence the fluctuating excitability of the forebrain rather than to transmit patterns of signals. The functions of this internal core have to do with the performance of tasks involving response chaining.

Pribram (5) makes a further distinction within the internal and external systems of the forebrain: each has an extrinsic and an intrinsic component. The extrinsic components receive afferents from outside the forebrain and are conceived to have an input function. The intrinsic components do not receive afferents from outside the forebrain and are conceived to have a problem-solving function. In the thalamus, the four anatomical divisions are as follows. External extrinsic nuclei are the ventral nucleus and the geniculates; external intrinsic nuclei comprise the posterior group (the lateral and the pulvinar). Internal extrinsic nuclei are the anterior group and the intralaminar and midline group, each of which is said to receive a strong extrinsic projection from the hypothalamus, the midbrain, or both. Internal intrinsic is the medial nucleus which receives no outside afferents. The cortical projections of these areas are similarly divided: thus the postero-dorsolateral cortex is external; the frontomedio-basal cortex is internal. The primary sensory areas are extrinsic; the so-called association areas are intrinsic.

In support of these finer distinctions Pribram (5) cites evidence that anterior (internal) intrinsic lesions upset the scalloping of a monkey on a two minute fixed interval reinforcement schedule. The scalloping indicates that the animal delays its response until there is some chance of reward. Posterior lesions had no effect. Thus, the problem of associating reward with time is upset by internal intrinsic lesions.

In a different experiment, monkeys were to find which of several objects covered a peanut and then select that object consistently, wherever its position. The experiment appeared to show (a) that systematic searching ability is inferior in the animals with posterior (external) intrinsic resections; and (b) ability to select the object under which the peanut previously was found is inferior in the animals with anterior (internal) intrinsic resections. Thus, the posterior area has to do with systematically distinguishing objects from one another; the anterior has to do with associating objects or times with rewards.

A more differentiated but at the same time less complicated view of the same problems has been repeatedly stated and expanded by Penfield (6, 7, 8). Penfield is of the opinion that information comes in through the primary sensory systems to the neocortex where it is encoded and sent back to an integrating mechanism in the internal (reticular) systems of the thalamus and midbrain; thence a copy of the message apparently goes up to the paleocortex to be recorded as a memory trace and at the same time to scan older memory trace systems. When a relevant memory is found by this scanning process it is projected as a second message into the lower internal system. Some evaluation of the memory message in relation to the original occurs; and if they match, a program from the memory is selected which now is projected from the lower internal system up to the voluntary motor system of the cortex whence it is converted into behavior. This of course is a free translation of Penfield's notion and owes something to exegesis by the author and his colleagues.

MOTIVATIONAL SYSTEMS

It becomes increasingly clear that there are motivational systems in the brain differentiated from other structures, that these are divided again into regions yielding positive (10) and others yielding negative reinforcement (11) upon electrical stimulation, and that the regions yielding positive reinforcement are probably divided again according to basic drives so there is a sexual-reward system, a hunger-reward system, and others. All of these differentiations appear to have an anatomical basis (10). The areas in which electrical stimulation causes approach behavior include large sections of the rhinencephalic cortex in addition to related structures in the thalamus and other basal ganglia, most of the hypothalamus, and parts of the tegmentum. In general the same group of structures yields these effects in rats (12), cats (13), monkeys (14, 15, 16), porpoises (17), and human beings (18). Schizophrenic patients reported pleasure but could not specify it definitely. One patient of 25 who were tested reported sexual pleasure. It is also reported that in higher vertebrates stimulation of some areas of the neocortex yields

these effects (19), but these areas are few. Several investigators have reported failure of positive reinforcement by electrical stimulation of the septal area in cats or monkeys (13, 20). There is general agreement, however, that the other nuclei and tracts in the septal region (the nucleus of the diagonal band, the anterior commissure region, medial forebrain bundle) which give major self-stimulation effects in rats also give such effects in cats and monkeys (13, 21), and the habenular nucleus, which fails to give the effect in rats (12), also fails in cats (20). It is important to remember that the septal nuclei do not lie in the septal area of higher vertebrates (22).

That the approach motivation produced by electrical stimulation of certain areas varies with the strength of basic drives has been demonstrated (10, 23, 24, 25). It is also reported that the regions in which self-stimulation effect varies as a function of hunger drive are differentiated from those in which the self-stimulation response varies as a function of androgen level (26). The hypothesis is proposed, therefore, that there are hunger-reward systems different from sexual-reward and from other reward systems (10). On the other hand, it appears that forced electric stimulation in some hunger-reward centers causes animals to eat more (10, 27), and forced stimulation in sexual-reward centers causes erection (17, 28).

As to the question of whether the positive reinforcement produced by electrical stimulation compares with conventional primary rewards, it is shown that in the maze and the runway animals learn about as well for the electrical reward as hungry animals do for food (10). Further, in an obstruction box, animals will endure far more pain to secure a brain stimulus than hungry rats will endure for food (10). It is also shown that anxiety which deters a thirsty animal from responding to get water does not deter a self-stimulating animal from his endeavors in pursuit of electrical stimulation (14), if the electrodes are in certain parts of the septal area.

Several investigators have reported reward and punishment from the same electrode placement (17, 28 to 33). For the most part these reports involve electrodes placed so that they stimulate a region between a dorsal negative-reinforcement system and a more ventral positive-reinforcement system in the hypothalamus and tegmentum. In these same regions (but not in others) it is shown (10, 34) that, as the stimulating current is increased, the self-stimulation rate may first rise, then decline with increased current, and rise again with further current increases. The rather rare decrements in self-stimulation rate when voltage is increased and the reward and punishment from the same electrode placement are both explained by the mixed regions in which the electrode is planted. One electric field invades two anatomically differentiated systems. When electrodes are planted in regions far from negative reinforcing systems, there are no such conflicting effects, and all increases in electric current produce increases in response rate up to a certain asymptote characteristic of the region (10).

That "ambivalence" can result from stimulation of two different areas with one electrical field is clearly shown by Lilly using roving electrode techniques (15, 35). It is also reported that when electrodes are in self-

stimulation areas which also produce erection, there is a tendency for animals to start the stimulus if it is off, but also to stop it if it is on (19).

It is determined that with electrodes in hypothalamic areas, self-stimulation does not shut itself off by some satiation mechanism. When animals were allowed to stimulate themselves continuously for 48 hr., those with hypothalamic electrodes showed no tendency to slow or to stop until the animal collapsed from fatigue. When electrodes were in telencephalic placements, however, satiation was evident; after 4 to 8 hr. of continuous self-stimulation, animals stopped completely or slowed markedly (10).

Other major effects achieved by electrical self-stimulation at almost all effective points are: (a) parasympathetic autonomic effects (36, 37); (b) excitation and inhibition of locomotor behavior (38); and (c) the sexual or eating effects mentioned above (10, 17, 27, 28). These correlations have led to the suggestion (39) that areas in the brain which are sensitive to hormonal messages indicating need states respond by producing immediate autonomic effects to release bodily stores, on the one hand, and by selectively facilitating and inhibiting learned behavior patterns to replenish bodily stores on the other. Behavior programs previously terminated by rewarding effects would later be released into action by the facilitatory influences emanating from these systems. Rewarding stimuli which arrest or quiet the animal act by means of the inhibitory influences emanating from these systems. This entire positive motivational system is considered to be different from a more primitive (negative feedback) avoidance system housed mainly at the midbrain and dorsal posterior hypothalamic level.

LEARNING MECHANISMS

The question of the nature of learning mechanisms in the central nervous system is still almost totally unsolved. The problem of how to begin a research on these mechanisms brings up the question of whether learning occurs diffusely in the whole central nervous system or whether it (or at least some central aspect of it) is a focalized process that can be somehow localized. The grossest point of view is that growing, perhaps, out of Lashley's work (40), but best expressed by Hebb's neurobiotactic conception (41): wherever two neurons meet, learning is a distinct possibility. Whenever an impulse in an axon of A successfully fires B, an increment of facilitation occurs so that A is more likely to fire B in the future. At the other extreme is the view held by Penfield (42) that among the other apparatuses of the brain, some of which accept the input, encode it, scan, integrate, activate, and so forth, there is also an apparatus which acts essentially like a wire recorder to put down the stream of consciousness in some more or less discrete location as one long strand holding the imprint of experience from birth to death. In between these two extremes run intermediate possibilities. One has stemmed largely from the ablation experiments after Lashley's work: the conception that different learnings are localized in different places. Pribram's (43) conception of a discrimination learning mechanism in the posterior intrinsic systems and a motivational learning mechanism in the anterior intrinsic

systems is just one example. A final possibility which cannot be entirely overlooked was suggested by Ashby (44) and turns for data to experiments which appear to show learning in single-cells or single celled organisms. The possibility is that all cells have the property of adapting output in a given environment, so as to maximize unit response patterns which produce positive reinforcing and avoid negative reinforcing inputs to the unit itself. The environment of a nerve cell would be the chemical environment and the pattern of discharges on it. Ashby suggests that each cell is basically a negative feedback mechanism. Such a possibility seems at first to commit one to the "learning occurs all over" view; but one has only to remember the specialization of certain cells to certain aspects of cellular function, as long (nerve) cells are specialized to conduction, to realize that learning still might be localized anatomically according to this view.

As to current research, we will consider: (a) the evidence for the likelihood that a successful synapse extends its own synaptic power; (b) the evidence from electrical stimulation, recording, and ablation for and against a "learning center" in the brain; (c) attempts to analyze the learning mechanisms into a series of more or less independent processes with discrete localizations for the different sublearning categories; and finally (d) information on quasi "purposive" adaptations of single cells.

Starting with what was called posttetanic potentiation (45) and is now called postactivation potentiation (46), the phenomena in question are twofold. First, after tetanic stimulation of particular afferents of a ventral horn unit, there is a temporary facilitation of the synapses involved so that the postsynaptic potential is greater in response to a single volley, and the postsynaptic unit is more easily fired. The potentiation in these cases lasts for extended periods up to five minutes and even longer, provided the test is made with a single shock stimulus; with trains of test stimuli, the potentiation declines rapidly. Second, (45) there is the loss in excitatory power by unused afferents, which can be restored by tetanic volleys. In these experiments sensory fibers of the cord are deafferented but axons remain in connection with cell bodies. After a period of disuse these fibers are used to stimulate ventral horn cells. The postsynaptic potentials excited by their stimulation are far below those of control. This depression can, however, be immensely restored by tetanic stimulation of these dorsal roots, and the restoration has an even longer time course than that mentioned above. As the Hebb theory of neurobiotaxis would demand, these studies show the potentiation to be specific to the synapses involved in the tetanus and not generalized to other afferents of the same postsynaptic unit.

According to Eccles' present interpretation of the phenomenon of potentiation (46), it derives from changes in rate of the process whereby the presynaptic unit makes and stores the transmitter substance at the synapse. When the unit is tetanized, some aspect of this process is speeded up and thus more transmitter becomes available at the synapse, causing the larger response to each volley. Under disuse this process slows down, causing absence of transmitter at the synapse and a small response to each volley.

Through this interpretation, and the fact that the potentiation occurs whether or not the postsynaptic unit discharges, it is clear that this is not a model for Hebb's learning mechanism. For Hebb's (41) theory requires that only successful synapses be potentiated; and in the case of posttetanic potentiation, all synapses that are even tried by an afferent volley (whether they succeed in firing efferents or not) are potentiated. Nevertheless, this is a demonstrated plasticity of the central nervous system of considerable duration. It cannot be overlooked at present in our search for an interpretation of learning because so few phenomena with adequate time span have been demonstrated thus far.

Turn now from the possibility that every synapse is a mechanism of learning to the current pursuit of Lashley's (40) will-o'-the-wisp, the site of the engram, the learning center of the brain. This year's candidates have included the midbrain reticular system (47, 48), the thalamic reticular system (49, 50, 51), the caudate nucleus (52), the interaction of sensory afferent and efferent systems (3), and last (but no longer taken lightly) a system originating in the hippocampus and projecting into the septal, habenula, and perhaps also mammillary nuclei as suggested by Penfield (6, 8). A technique rapidly coming to compete with lesions and ablations in this sphere is the attempt to disrupt temporarily the supposed learning mechanism by electric stimulation (47, 49, 51, 53, 54). This provides a reversible lesion.

Two studies seem to point to the reticular formation itself as crucial in some part of the process in some kinds of learning. First, a study from Hebb's laboratory by Glickman (47) indicates that rats stimulated in some part of the midbrain reticular formation, just after receiving a heavy shock at the food cup, completely forget the experience just-preceding so far as the measures used could determine. This result was interpreted according to a consolidation period hypothesis: a reverberating circuit including reticular units was considered to hold the information until a more permanent record could be made. Second, the work of Sharpless & Jasper (48) mentioned in last year's review (55) indicated that learning to discriminate between an important and an unimportant tone (adaptation) could occur without any of the higher auditory apparatuses, and the assumption was that it occurred at a reticular level of midbrain or diencephalon.

Work by Mahut (49) from Hebb's laboratory pertains to the thalamic reticular formation. Stimulation was applied in intralaminar and midline thalamic nuclei after each run in a maze. The group receiving stimulation did significantly worse than a group receiving no stimulation. The work of Ingram, Knott & Correll (50) substantiates the view that stimulation of diffuse systems in the thalamus has some major effect at least on efficiency of performance. These investigators used a bar-pressing technique with which they showed that animals stimulated in the thalamus worked more slowly than animals without stimulation.

Rosvold & Delgado (52) have reported that stimulation in the caudate nucleus impaired performance of monkeys on a learning set problem, but

that no impairment resulted from stimulation with nearby electrodes outside the caudate. These authors also report that bilateral lesions in the same regions cause similar defects.

As to the structures of the fornix system (hippocampus, septal nuclei, habenula, and mammillary body), Correll (53) has failed to demonstrate effects of hippocampal stimulation in the cat on what he calls learned behavior (it appears, however, that he measured not learning but speed of performance). Data on the positive side come from clearcut results by Thomas & Otis (56) indicating that lesions invading the rostral tip of the hippocampus, presumably cutting off its downward projections, obstruct avoidance learning, as previously indicated by Pribram & Weiskrantz (57), as well as approach learning (58) in a maze. Also, Stein (59) has indicated that electrical stimulation on the boundary between the hypothalamus and the septal region, in areas from which self-stimulation can be obtained, causes an impairment in discrimination learning.

In other studies (54), electrical stimulation in certain periamygdaloid, hippocampal, septal, and epithalamic or habenular placements has caused a reversible change to chance level performance of rats on a learning set problem. No major effect on speed of performance or on eating behavior in the problem box was produced. Stimulation in caudate regions and sometimes in some parts of the diffuse thalamic system causes a reversible loss in ability to perform at all [cf. (60)] or a change in rate of performance without materially affecting errors. Stimulation in other areas causes reversible decrement in eating. In these experiments (54) sixty cycle sine wave stimulation is applied in a continuous series of trains (0.5 sec. every second or 0.5 sec. every three seconds) and is varied from 10 to 50 μ amp. On experimental days, stimulation is applied; on control days it is not. The animal serves as his own control and, each day, solves anew the problem of finding which lever activates a food magazine. It is only the areas where stimulation obstructs errorless performance without affecting speed of running or eating behavior that are considered as being possibly involved in a system differentiated for mechanisms of learning.

Orbach, Milner & Rasmussen (61) have removed hippocampal areas in monkeys in an effort to confirm the deficit in short term memory reported by Milner & Penfield (62) in humans. So far this particular effort has met with little success.

In regard to efforts to correlate learning factors with cortical areas, Pribram (43), previously mentioned, reports two experiments suggesting that frontomedial lesions affect reward-associations and that posteromedial lesions affect solution of discrimination problems. Konorski (63, 64) presents data showing that ablation of frontal lobes in dogs produces marked deficits in delayed response and in ability to learn inhibitions in a situation where A initiates a response but the addition of B (to make A and B) inhibits the response in the intact animal. Mishkin (65) reports that lesions of the midlateral frontal granular cortex produced most severe deficits in delayed response problems. Mishkin & Weiskrantz (66) also showed that ani-

mals with frontal lesions were less able to tolerate longer intervals of delay of the reward after problem solution than controls.

Work on the electrical correlates of learning has advanced rapidly but has left much to be desired because of the difficulty of distinguishing between electrical correlates of the conditioned response and electrical correlates of learning itself. Most of the reported work appears to give more information on the former than on the latter. Jasper, Ricci & Doane (67) have directed their attention frankly to this problem in a chronic microelectrode investigation. They have shown that in the motor cortex it is possible to see unit discharges evoked by the conditioned stimulus which precede the actual elicitation of the limb movement which is being conditioned. In this case, the obvious importance of the technique suggests a more definitive path for study of correlates of learning in the future, permitting, as it must, microanalysis of unit discharge in the brain during cognitive and performance processes. Strumwasser (68) also reports chronic recording of unit discharges with implanted microelectrodes.

As to other electrical changes in the brain associated with learning, Galambos (3) has given the following classification. (a) Blocking of the alpha rhythm (or other slow rhythms) may be conditioned. Here a stimulus which causes arousal is used as unconditioned stimulus, and a stimulus which no longer causes arousal is used as conditioned stimulus; after pairing the two the conditioned stimulus causes blocking. Rowland (69) and Yoshii *et al.* (70), have reported such experiments this year. As mentioned in last year's review (55), Morrell & Jasper (cf. 71) have also reported work of this sort. (b) New waves may be evoked. During some processes Morrell (71, 72), Lesse (73) and others have observed sudden occurrence of activity at roughly 40 cycles per sec. from cortex and perhaps, particularly, from amygdaloid regions (a rhythm which Lesse has thought to be correlated with thought processes). (c) Evoked potentials may be protected from attenuation. Galambos (3) himself has shown that responses to click in the cochlear nucleus are attenuated by attention in other directions [as reported previously by Hernandez-Peón (74, 75)]. After habituation, too, the response to click becomes labile (undependable). Then after some clicks are paired with shocks, the response ceases to be labile and becomes dependable and the size of the cochlear response is significantly augmented.

In a further experiment, a cat is first adapted to a series of 11 clicks. After habituation, the animal is shocked at the eleventh click. The new appearances in the record after shock involve: (a) occurrence of new waves at 38 to 40 per sec. during the 11 clicks in the globus pallidus and the amygdala and sometimes from subcortical leads; and (b) an impressive increase in amplitude of evoked potentials in cortical and subcortical leads.

By averaging a series of evoked potentials at each stage of the experiment, Galambos was able to show impressive acquisition and extinction curves during the conditioning procedure. Finally, comparing responses in the conditioned and extinguished states, he found that after extinction the response to the stimulus was relatively small in amplitude and short in

duration. The conditioned state, on the other hand, was associated with large responses lasting a long time.

Two interesting experiments specifically followed the procedure outlined in the work of Morrell (71) and Jasper (94) employing a flicker in the alpha range as unconditioned stimulus, and a tone as conditioned stimulus. Yoshii *et al.* (70) using this technique reported results almost identical to those of Morrell and Jasper. In the first stage of conditioning, evoked potentials and a desynchronization appear during the presentation of the conditioned stimulus; during the second stage the desynchronization is replaced by hypersynchronous repetitive discharge which progressively 'ends to follow the frequency of the intermittent photic stimulation. Frequencies of the photic driving range appear in the reticular leads as the animal is placed in the test box, even when there is no conditioned stimulus presented.

In similar studies Chow, Dement & John (76) sought to determine whether the provocation of the photic response by the tone had somehow rendered the two stimuli equivalent. They first trained a behavior to the photic stimulus, then carried out the Morrell and Jasper type of conditioning of tone to photic stimulus until the tone evoked the rhythmic discharge, and then tested the animal for behavioral response to the tone and found that it was absent.

John & Killam (77) have used photic driving differently, employing intermittent photic stimulation as conditioned stimulus (rather than unconditioned stimulus); the "following" response recorded from various brain areas then provides a "tracer" to show which regions of the brain are affected by the conditioned stimulus at different stages in the learning process. The results seem to show that, as a neutral stimulus, which originally produces a response only in the geniculate, first becomes associated with a shock, the geniculate wave disappears and septal-amygdaloid-reticular "following" begins. Then as a full-fledged conditioned emotional response develops, "following" moves to the hippocampus. Finally, as an avoidance response is learned, stimulus-following responses again become limited to the geniculate and the neocortex.

John & Killam (77, 78) have also reported an interesting series of tests in which a response is conditioned to occur for food under a 10 c.p.s. flash, and to inhibit at a 6 c.p.s. flash. The most interesting result occurs if the experimenter forces a mistake by giving a long series of 6 c.p.s. flashes followed by a 10 c.p.s. flash. At this point the 10 c.p.s. (from the environment) appears in the reticular formation and neocortex, and the 6 c.p.s. (presumably immediate memory to which the animal is responding) appear in the hippocampal and thalamic reticular leads. When the animal switches from mistake to response, the hippocampal following wave disappears and the thalamic reticular formation's rhythm goes into synchrony with that of the midbrain.

On the question of whether learning can occur within individual cells, primary interest has centered on the controversy over learning in paramecia reported by Gelber (79, 80). She indicated that paramecia which did not

originally approach a sterile platinum wire would later approach it after being trained to feed upon it. According to critics (81, 82, 83) it appears likely that the result occurs in some cases because of residual food in the part of the liquid environment where the needle is lowered. On the other hand, a number of studies cited in an excellent new treatise *Learning and Instinct in Animals* by Thorpe (84) makes it appear likely that, through use of other experimental procedures, it may be possible to demonstrate primitive associative learning in single-celled organisms. The possibility cannot be overlooked that the tendency in the past to reject out-of-hand evidences of learning in single-celled creatures has derived from a too early prejudging of the issue in terms of learning as a charge at the synapse.

Three new items of further interest may be mentioned at the end of this section, even though they do not coincide with any of the particular points being made. First is the new set of observations derived from Loucks' (85) classical experiment with stimulation to one part of the cortex as conditioned stimulus, and stimulation to the motor cortex as unconditioned stimulus (86, 87, 88). It is now found, contrary to Loucks, that pairing of neutral conditioned stimulation with motor cortical stimulation eventually causes the neutral stimulus to evoke the motor response. Loucks' original failure to obtain these results is thought to be a result of too rapid repeat frequency of the unconditioned stimulus, which causes inhibition to build up. Second is the publication on a long line of "imprinting" experiments by Hess (89). This work shows that there is definitely a crucial and brief period in birds for imprinting to occur and (a) that the degree of this learning is a function of the effort output during initial following (training); (b) that the lack of learning after the crucial period is not offset by an anxiety-reducing drug (meprobamate in fact causes poorer imprinting at all times). Some of the same points are also made in experimental work by Jaynes (90, 91) and also in Thorpe's new text (84). The third and final item is the demonstration by John, Wenzel & Tschirgi (92) that reserpine acts selectively on some kinds of learning as opposed to others. They trained cats to avoid both a visual and an auditory stimulus and found that reserpine abolished visual avoidance but not auditory avoidance. Then they trained animals to obtain food in a pattern discrimination box and to avoid shock in a hurdle box. Reserpine suppressed the avoidance response without affecting the approach. In cases where avoidance was obstructed by reserpine there was evidence of a conditioned emotional response to the conditioned stimulus, and there was adequate escape during the drug tests. Therefore, these investigators concluded that reserpine acts selectively on some of the learning connections involved in the performance of avoidance responses.

MECHANISMS OF INTEGRATION

This year the mechanisms of integration have taken on more clearly defined status, both anatomically and functionally. That is, a better understanding of the anatomical regions dealing with integrative problems and of the physiological control and measurement of integrative functions has developed.

Reticular formations.—The reticular formations of the midbrain and thalamus have previously been assigned diffuse projection roles upstream to the cortex and downstream to the cord. Also, they have been seen to receive afferents from all of the receptors and from the cortex (93). Thus, they receive from and project to almost all parts of the brain. These formations have been divided into caudal or midbrain parts which appear to handle more general facilitating and inhibiting functions, and rostral or thalamic parts which have more specific control, selecting certain patterns for attention and others for occlusion (94). In the direction of the periphery (95, 96, 97) they have been shown to control not only amplitude of motor reflex phenomena but also to gate incoming sensory activity so that activity in the dorsal columns of the spinal cord and potentials evoked in the cochlear nucleus are modulated by general or specific messages originating in reticular regions (98). In the direction of the cortex, they have been shown to cause the activation or inhibition of individual cortical neurons (99 to 102) and to modulate the responsiveness of some of these to incoming primary sensory signals (103). Also, in the cortex they have been shown to have diffuse control over the slow wave changes attributed to the dendritic layer (104). (The dendritic layer of the cortex which itself may be regarded as another integrating mechanism is discussed later.) It has been shown that these formations receive afferents from relatively specific regions of the neocortex (105), those regions previously thought of as suppressor strips, and from certain parts of the paleocortical mantle.

Microelectrode recording from individual units in the reticular formation has shown a wide variability in the afferent fields of such units so that some respond to a wide range of stimulations and modalities (106, 107); others respond to a much smaller range of stimulations (to the extent that it almost appears that some units might be differentially responsive to specific sensory inputs). Most interesting, perhaps, as a result of this microelectrode analysis is the suggestion (107) that units excited by more than one modality of stimulation respond with a pattern characteristic of that modality so that one temporal pattern of firing would announce stimulation from one modality, another pattern would announce input from another. Incidentally, a similar finding has been reported for the geniculate: cells giving "on" response to one of two complimentary colors may give "off" response to the other (108). Thus where the localization code breaks down there may be a pattern of firing code to carry information.

Another exciting outcome of reticular study this year is the behavioral confirmation of the hypothesis of the Magoun group (93) that reticular activation is a neural correlate of attention. Fuster (109) has shown that the efficiency or speed of perceptual discrimination can be reliably increased in the monkey by stimulation of the reticular activating system. Further behavioral effects of reticular stimulation, obtained from all points tested in the rat, include rewarding or punishing effects, or both (30, 31). There is also a curiosity-attention, fear, terror sequence produced by increasing stimulation of reticular-projecting cortical points (105, 110). Finally, the work of a group in Japan (111) throws light on the problem of sleep or wak-

ing evokation by thalamic reticular stimulation. This study demonstrates that high frequencies cause the animal to wake and low frequencies of recruiting range cause animals to sleep.

Cortex.—Turning to the integrative mechanisms of the cortex, we find topologically discrete integrating systems (those that activate the reticular activating system) (105) and an architectonically differentiated layer, the dendritic mantle over the whole cortex which appears to carry voltage patterns for some time that are related to differential excitations and inhibitions of corticopital units (94, 112). The topological areas which act on the reticular formation are sensorimotor cortex, frontal oculomotor fields, paraoccipital region, anterior cingulate gyrus, superior temporal lobe, and the tip of orbital surface of frontal lobe (105). Stimuli applied to any of these areas cause evoked potentials through the whole reticular formation with no differentiating characteristic of any one of them vis-à-vis the others. The impulses from these areas appear to funnel through the septal nuclei and perhaps thence through a hippocampal, entorhinal path to the reticular areas. They cause not only evoked potentials in the reticular formation, but cortical activation (as from reticular area) and behavioral arousal as from reticular stimulation. Other experiments show a profound cortical inhibition on downstream motor activity provoked via the cortex by stimulation of the reticular activating system (95, 96, 97).

As for the architectonic layer, the dendritic plexus of the cortex appears to be the site of generation of slow wave activity recorded by d.c. methods (113). Sensory stimulation causes major slow potential changes to be recorded from this region; stimulation in the reticular or thalamic reticular groups also causes slow wave shifts. More detailed studies (114) appear to indicate that these potentials are generated postsynaptically in the cortex. D.c. shifts appear to be partly correlated with arousal in the sense that arousing stimuli produce the shifts; but they are separable in that arousal can continue after d.c. shift has gone, and d.c. shift can be superimposed on background of arousal. Slow potentials generated by sensory and midbrain reticular stimulation can be abolished by small doses of pentobarbital which do not affect the evoked potentials of specific projections. These doses do not abolish the thalamic reticular effects. The d.c. phenomena evoked by 60 per sec. stimulation in the thalamic reticular are more localized than those produced by sensory or reticular stimuli; they are limited to the same areas to which recruiting responses are localized.

Freeman (115) appears to have confirmation for a view that at least some of the normal EEG waves may be generated in the apical dendrites (as battery), fed down axons into the thalamus as d.c. flow (to be differentiated from the spikes taking the same route but conveying different information), and then circuiting back up through the intercellular space to the cortex to complete the circuit. He calls this route a "cortico-medial dipole." This view together with the illustrated control over dendritic slow potentials by reticular stimulation appears to extend the idea suggested by Jasper (94) and Grundfest (112) that the reticular formation operates in consort with, or against the background of, the dendritic meshwork to modulate its more en-

during potentials, and together these two controls perform an integrating function on output from the cortex. Jasper suggests that so-called "activation" is a patterning of excitation and inhibition fed from the reticular formation up into the more sustained excitatory or inhibitory patterns held in the dendritic meshwork. Grundfest suggests that in this pattern of excitation and inhibition in the dendrites, which controls spike discharges in axons of the same units, there is interaction between mechanisms serving as computers, one group of the more flexible analogue type, the other of the more precise digital type.

Functional studies.—If a functional approach is now adopted some of the material may be repeated briefly, but the alternative mode of analysis also allows introduction of other materials. The integrative mechanisms actively studied this year include: (a) the interaction of excitation and inhibition (94 to 97, 116); (b) the waves of the brain that are recorded by EEG and d.c. potential techniques (113, 115, 117); (c) the interaction of modalities or of diffuse and specific systems on the firing of single units (103, 107); and (d) the correlation of neural activity with blood flow and neurohumoral mechanisms (118, 119, 120).

Some of this material has been mentioned above. Concerning the nature of excitation and inhibition, both Purpura (116) and Jasper (94) have cited new experiments which indicate that γ -aminobutyric acid (GABA) inhibits the excitatory (surface negative) response of dendrites and permits a formerly unseen inhibitory (surface positive) wave to appear. As the cerebellar cortex is believed to have relatively few inhibiting units (121), it provides a control for these findings and Purpura shows that, in the cerebellar cortex, GABA inhibits the excitatory component without revealing any inhibitory wave. This provides strong support for the argument of Grundfest (112) and Purpura (116, 121) that the surface positive wave is an inhibitory potential of dendrites, and the surface negative wave is excitatory in the same region.

Concerning control of neural activity by potentials in the EEG range, Lindsley (117) shows alpha waves to have some influence on responsiveness. Schlag's studies (122) involving microelectrode recording show that some units have discharges correlated with slow wave activity; others are uncorrelated. In the rostral thalamic reticular nuclei and in the primary sensory nuclei of the thalamus unit, activity is correlated with slow waves. But in the midbrain and posterior centre median (caudal reticular formation) the activity of units is not correlated with slow waves.

Dell (118) shows the reticular system to be an epinephrine-sensitive receptor, among other things, so that intravenous injection of epinephrine activates the cortex if it is connected to the reticular formation; if a section is made between the cortex and reticular area no similar activation is found. On the other end there is downstream activation from epinephrine if the cord is in connection with the reticular system. The work of Rothballer (120) shows the caudal reticular system [which Jasper (94) identifies as controlling tonic longer-lasting general activation] is sensitive to epinephrine; the rostral reticular system (which Jasper sees as phasically controlling

specific admissions and exclusion of stimuli) is not sensitive to epinephrine. The work of Ingvar (119) shows that cortical activation is correlated with the rate of blood flow.

CHEMICAL FINDINGS

Major work has centered around what might now be considered competing hypotheses as to the roles of 5-hydroxytryptamine (serotonin) versus epinephrine as transmitters with important function in the etiology of mental disorders. Ever since Osmond & Smythies (123) indicated the similarities between mescaline and epinephrine and followed this with the apparent demonstration that adrenochrome (an oxidation product of epinephrine) might cause psychosis, there has been investigation of the possible role of faulty epinephrine metabolism in psychosis. Repetition of the Osmond and Smythies tests (124) failed to confirm the notion of psychosomimetic effects, but the list of epinephrine oxidation products has grown so the hypothesis is not so easily pinned down. Adrenolutin, adrenoxine, and others are now suggested also as possible psychosomimetic products of epinephrine (125).

The evidence concerning serotonin is of a different nature. Woolley & Shaw (126), Gaddum (127), and Brodie & Shore (128) have come to suggest different theories of psychosis based on serotonin because of the actions of lysergic acid diethylamide (LSD) on the one hand and reserpine on the other.

Woolley & Shaw (126) suggest that LSD causes mental disturbance by either depleting the quantity of serotonin in the brain or by inhibiting amine oxidase (which breaks down serotonin) thus permitting accumulation of serotonin in the brain. [It is shown by Hoagland's laboratory (125) that LSD inhibits the destruction of serotonin by amine oxidase.]

This view of the connection between serotonin and mental disorder derives first from the finding that serotonin causes smooth muscle to contract (126, 127) and that antagonists of this reaction (harmine, yohimbine, ergot alkaloid, LSD) have psychiatric effects, second from the fact that serotonin occurs in "emotional" regions of the brain (127, 129), and third from the fact that oligodendroglia of the brain in tissue culture are caused to contract by serotonin, a reaction which antiserotonins (like LSD) either antagonize or mimic (126). (Woolley & Shaw suggest that the same compound can either promote or antagonize serotonin action depending on the test and concentration used.) From this evidence it is proposed that upsets of serotonin levels (or actions) are at the root of psychotic episodes caused by antiserotonins. This notion has instigated a search for techniques to show that serotonin can counteract LSD effects in the brain. Loading an animal with serotonin does not cause any excess to be found in the brain; either it is oxidized immediately by monamine oxidase or it fails to cross the blood-brain barrier (126). Direct intracerebral injection in the mouse has been tried but it has failed to block the LSD effect (126).

Olds, Killam & Eiduson (130), however, demonstrated a direct antagonism in the brain. LSD caused self-stimulation to cease for an 8- to 20-min. period. Preloading by thirty minutes with serotonin intraperitoneally pro-

tected about one-half of the animals from this LSD effect (animals which were not protected were also the only animals in which LSD and brom-LSD had similar effects).

Rothlin (131), however, by showing that brom-LSD, which has the same antiserotonin properties as LSD, causes no psychic changes, indicates that the antiserotonin action of LSD may not be at the root of its psychosomimetic effects. [Brom-LSD appears to cross the blood-brain barrier as easily as LSD itself (131).]

Brodie & Shore (128) start from the fact that one action of reserpine is to impair the serotonin-binding sites so that they lose in part their capacity to retain serotonin. As a result, serotonin formed in the brain, instead of being stored, remains in a free active form; the brain thus is presented with a persistent low concentration of free serotonin. To this now free serotonin they attribute excitatory properties in the parasympathetic system and say this parasympathetic activation is at the root of reserpine's tranquilization. Reserpine in low doses has been shown to facilitate rhinencephalic seizures (132). It should be noted, however, that serotonin when unbound is rapidly converted by monoamine oxidases to 5-hydroxy-indole acetic acid and secreted in the urine so that brain levels of serotonin assayed several hours after reserpine injection are far below normal (133). One might expect this relative absence of serotonin to account for reserpine effects rather than the free state of the little which is left [cf. (134)]. In either case, the long-lasting changes in serotonin levels caused by one dose of reserpine seem to account for the long-lasting effect of this chemical, which has its major action after brain levels of the drug have reached vanishing proportions (128).

Brodie & Shore (128) go on to a further paradox: if reserpine is given but a monoamine oxidase inhibitor, iproniazid, is given previously, reserpine does not cause the brain levels of serotonin to fall; here there is free serotonin and now it remains in the brain. In this case reserpine does not have its ordinary sedative effects (on the rabbit) but seems to activate. Brodie following the same logic as above holds that this overdose of serotonin has antagonized serotonin effects (as too much acetylcholine has been shown to antagonize acetylcholine effects) and, therefore, the animal is activated. In this case there are some data (135) which show that loading a dog with a serotonin precursor, which increases brain levels of serotonin, also causes an "activation" similar to that of LSD.

Brodie contrasts the action of reserpine with that of chlorpromazine: reserpine tranquilizes by freeing serotonin, the transmitter which activates the parasympathetic (quieting) system (136); chlorpromazine, he says, tranquilizes by blocking the action of norepinephrine, the transmitter which activates the antagonistic sympathetic (agitating) system.

Chief difficulties for this theory as it stands are: (a) the fact that reserpine frees and depletes brain levels not only of serotonin, but also of epinephrine and norepinephrine, the concentration of which in the brain evidently follows the same series of changes as that of serotonin after reserpine injections (132, 137, 138); (b) the fact that the monoamine oxidases also serve to break down norepinephrine and epinephrine as they do to serotonin—so the

mentioned effect of iproniazid may result from potentiation of any of three amines (serotonin, epinephrine, or norepinephrine) (132); (c) the fact that epinephrine and norepinephrine are always found in the same regions of the brain as serotonin, in proportional concentrations (139); and (d) the fact that self-stimulation in parasympathetic regions of the hypothalamus is blocked completely by chlorpromazine (10, 130) in doses which have no major effect on avoidance of stimulation in sympathetic regions of the hypothalamus (140). The inhibition of parasympathetic self-stimulation by chlorpromazine is paralleled by its inhibition of sexual behavior (141).

Pertinent also to the theory, but not directly for or against, is the fact that serotonin injected directly into the hypothalamus (after preinjection of iproniazid down the same pipette) causes collapse of self-injection behavior and profound depression of the whole animal; epinephrine injected now against this background of depression restores the animal to a generally alert and active condition and restores self-injection behavior. This occurs when pipettes are implanted in parasympathetic regions of the hypothalamus where rewarding effects of chemical stimulation have been observed (142). These data must be viewed together with those of Dell (118) indicating an epinephrine receptor in the reticular formation, and those of Elkes (143) and Bradley (144) indicating direct excitation of the reticular formation by the monoamine oxidase inhibitor amphetamine.

On the basis of these newer data it appears possible that epinephrine or norepinephrine is the excitatory transmitter in both the sympathetic and the parasympathetic systems, serotonin perhaps the inhibiting transmitter in both systems. The action of reserpine is perhaps to deplete both inhibitory and excitatory transmitters, thereby causing sedation of the system; the action of chlorpromazine may be to block the excitatory action of epinephrine. Iproniazid then potentiates both epinephrine and serotonin but has the effect of the excitor. This would follow from the formulation, excellently set forth in Eccles' (46) new book, that inhibitory transmitters render cell membrane selectively permeable to ions as large as potassium but not as large as sodium; excitatory transmitters render membrane permeable to both the smaller and the larger ions. In such a case the transmitter causing permeability by the larger ions would predominate in case of a joint action of unbound excitor and inhibitor at the same time.

The pharmacological data from self-stimulation and self-injection experiments may be elaborated also in the direction of a better understanding of psychotic agitations and depressions: (a) since the effective tranquilizer chlorpromazine acts selectively against the positive motive systems of the hypothalamus (10); (b) since meprobamate (which is not effective against psychotic agitations) has no similar action against positive motive systems (on the contrary, it releases some from major inhibitions) (145); and (c) since iproniazid, which acts against psychotic depressions, possibly activates these same positive systems (142); it therefore appears likely that excessive activity in positive motive mechanisms of the parasympathetic hypothalamus is at the root of psychotic agitation, and insufficient activity of these same mechanisms is at the root of psychotic depressions. The notion that psychotic

states owe much to positive motive systems is rendered plausible by the runaway behavior demonstrated in these "positive feedback" systems (146) which indicates that they need always be subject to strong external controls.

CONCLUSION

At each stage in this rapidly progressing quest for understanding, a gambler's urge tempts us to guess at more than we have found. On the basis of data available in June, 1958, I would guess that a major axis of differentiation in the central nervous system divides it into an interacting set of laminar systems that might roughly be identified at the rostral end by their forebrain components. There seems to be a primary sensory system devoted to selection and coding, an hippocampal-fornix-epithalamic system devoted to learning and memory, and an amygdaloid-stria-terminalis-hypothalamic system devoted to motivation. These all seem to feed information into and receive messages from a three-stage reticular core—comprehending midbrain reticular system, diencephalic reticular system, and septal nuclei. The septal nuclei appear to integrate higher functions (motivational and memory mechanisms); the midbrain seems to integrate sensory functions (input from the peripheral receptors and analyzed messages from the sensory cortex); and the diencephalic components may provide for integration as between the higher and the sensory functions. Then, following Penfield's suggestion, an integrated program from the reticular core might elicit behavior via the pyramidal system. Let the fact that the hippocampus changes function with each new experiment set the tone of tentativeness in this formulation.

LITERATURE CITED

1. Weinstein, S., and Teuber, H. L., *Science*, **125**, 1036 (1957)
2. Holmes, J. A., Teuber, H. L., and Weinstein, S., *Science*, **127**, 241-42 (1958)
3. Galambos, R., in *Central Nervous System and Behavior* (Brazier, M. A. B., Ed., Josiah Macy, Jr. Foundation, New York, N.Y., in press)
4. Pribram, K. H., in *Behavior and Evolution* (Simpson, G. G., Ed., Yale University Press, New Haven, Conn., in press)
5. Pribram, K. H., in *Handbook of Physiology* (American Physiological Society, Washington, D. C., in press)
6. Penfield, W., *J. Mental. Sci.*, **101**, 461-65 (1955)
7. Penfield, W., *Brain*, **77**, Part I, 1-17 (1954)
8. Penfield, W., in *Ciba Foundation Symposium: Neurological Basis of Behavior*, 149-74 (J. & A. Churchill, Ltd., London, Engl., 1958)
9. Olds, J., *Science*, **127**, 315-24 (1958)
10. Delgado, J. M. R., Roberts, W. W., and Miller, N. E., *Am. J. Physiol.*, **179**, 587 (1954)
11. Olds, J., *J. Comp. and Physiol. Psychol.*, **49**, 281 (1956)
12. Brady, J. V., in *Electrical Stimulation of the Brain: Subcortical Integrative Systems* (University of Texas Press, Austin, Tex., in press)
13. Brady, J. V., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 693-703 (Little, Brown & Co., Boston, Mass., 1958)
14. Lilly, J. C., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 705-21 (Little, Brown & Co., Boston, Mass., 1958)

16. Bursten, B., and Delgado, J. M. R., *J. Comp. and Physiol. Psychol.*, **51**, 6-10 (1958)
17. Lilly, J. C., in *Central Nervous System and Behavior* (Brazier, M. A. B., Ed., Josiah Macy, Jr. Foundation, New York, N. Y., in press)
18. Sem-Jacobsen, C. W., in *Conference on Electrical Studies on the Unanesthetized Brain* (Georgetown University Medical School, Washington, D. C., June 10-13, 1957)
19. Lilly, J. C. (Personal communication)
20. Doty, R. W., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 582-88 (Little, Brown & Co., Boston, Mass., 1958)
21. Olds, J., Killam, K. F., and Fuster, J. (Unpublished data)
22. Russell, G. V., Discussion after paper by Brady, J. V., in *Electrical Stimulation of the Brain: Subcortical Integrative Systems* (University of Texas Press, Austin, Tex., in press)
23. Brady, J. V., Boren, J. J., Conrad, D., and Sidman, M., *J. Comp. and Physiol. Psychol.*, **50**, 134-37 (1957)
24. Sidman, M., Brady, J. V., Boren, J. J., Conrad, D. G., and Schulman, A., *Science*, **122**, 830 (1955)
25. Nielsen, H. C., Doty, R. W., and Routledge, L. T., Jr., *Federation Proc.*, **16**, 93 (1957)
26. Olds, J., *J. Comp. and Physiol. Psychol.*, **51** (In press, 1958)
27. Wendt, R., and Olds, J., *Federation Proc.*, **16**, 136 (1957)
28. Miller, N. E., *Science*, **126**, 1271-78 (1957)
29. Roberts, W. W., *J. Comp. and Physiol. Psychol.*, **51**, 193-98 (1958)
30. Bower, G. H., and Miller, N. E., *Am. Psychologist*, **12**, 464 (1957)
31. Brown, G. W., and Cohen, B. D., *Federation Proc.*, **16**, 16 (1957)
32. Olds, J., in *Central Nervous System and Behavior* (Brazier, M. A. B., Ed., Josiah Macy, Jr. Foundation, New York, N. Y., in press)
33. Olds, J., and Peretz, B. (Unpublished data)
34. Reynolds, R. W., *J. Comp. and Physiol. Psychol.*, **51**, 193-98 (1958)
35. Lilly, J. C., *Science*, **127**, 1181-82 (1958)
36. Hess, W. R., *The Functional Organization of the Diencephalon* (Grune & Stratton, Inc., New York, N. Y., 180 pp., 1957)
37. Gloor, P., in *Hypothalamic Hypophyseal Interrelationships*, 74-113 (Fields, W. S., et al., Eds., Springfield, Ill., 1956)
38. Olds, J., in *Nebraska Symposium on Motivation*, 73-138 (Jones, M. R., Ed., 1955)
39. Olds, J., in *Biological and Biochemical Bases of Behavior*, 237-62 (Harlow, H. F., and Woolsey, C. N., Eds., University of Wisconsin Press, Madison, Wis., 1958)
40. Lashley, K. S., *Brain Mechanisms and Intelligence: a Quantitative Study of Injuries to the Brain* (University of Illinois Press, Chicago, Ill., 186 pp., 1929)
41. Hebb, D. O., *The Organization of Behavior* (John Wiley and Sons, New York, N. Y., 335 pp., 1949)
42. Penfield, W., in *Proc. Intern. Congr. Psychol.*, 14th Congr., 256 pp. (Montreal, 1954)
43. Pribram, K. H., in *Handbook of Physiology* (Hall, V. E., Ed., American Physiological Society, Washington D. C., in press)
44. Ashby, W. R., *Design for a Brain* (John Wiley and Sons, New York, N. Y., 259 pp., 1953)
45. Eccles, J. C., *The Neurophysiological Basis of Mind* (The Clarendon Press, Oxford, Engl., 314 pp., 1952)
46. Eccles, J. C., *The Physiology of Nerve Cells*, ix, 269 (Johns Hopkins University Press, Baltimore, Md., 269 pp., 1957)

47. Glickman, S. E., *Eastern Psychol. Assoc.*, 28th Annual Meeting (New York, N. Y., 1957)
48. Sharpless, S., and Jasper, H., *Brain*, **79**, 655-80 (1956)
49. Mahut, H., *Am. Psychologist*, **12**, 466 (Abstract, 1957)
50. Ingram, W. R., Knott, J. R., and Correll, R. E., in *Proc. Intern. Congr. Anat. of Vth Congr.*, 110-111 (Paris, France, 1955)
51. Ingram, W. R., in *(Henry Ford Hospital International Symposium*, 160-62 (Little, Brown & Co., Boston, Mass., 1958)
52. Rosvold, H. E., and Delgado, J. M. R., *J. Comp. and Physiol. Psychol.*, **49**, 365-72 (1956)
53. Correll, R. E., *J. Comp. and Physiol. Psychol.*, **50**, 624-29 (1957)
54. Olds, M. E., and Olds, J. (Unpublished data)
55. Masland, R. L., *Ann. Rev. Physiol.*, **19**, 533-58 (1957)
56. Thomas, G. J., and Otis, L. S., *J. Comp. and Physiol. Psychol.*, **51**, 130-34 (1958)
57. Pribram, K. H., and Weiskrantz, L., *J. Comp. and Physiol. Psychol.*, **50**, 74-80 (1957)
58. Thomas, G. J., and Otis, L. S., *J. Comp. and Physiol. Psychol.*, **51**, 161-66 (1958)
59. Stein, L., *Science*, **127**, 466-67 (1958)
60. Forman, D., and Ward, J. W., *J. Neurophysiol.*, **20**, 230-44 (1957)
61. Orbach, J., Milner, B., and Rasmussen, T., *Eastern Psychol. Assoc.*, 29th Annual Meeting (Philadelphia, Pa., April 11-12, 1958)
62. Milner, B., and Penfield, W., in *Proc. Am. Neurol. Assoc.*, 42-48 (Am. Neurol. Assoc., New York, N. Y., 1955)
63. Brutkowski, S., Konorski, J., Lawicka, W., Stepień, I., and Stepień, L., *Acta. Biol. Exptl. (Lodz)*, **17**, 167 (1956)
64. Lawicka, W., *Bull. acad. polon. sci. Classe VI: Serie sci. biol.*, **V**, No. 3, 107-10 (1957) (Presented by J. Konorski, March 7, 1957)
65. Mishkin, M., *J. Neurophysiol.*, **20**, 615-22 (1957)
66. Mishkin, M., and Weiskrantz, L., *J. Comp. and Physiol. Psychol.*, **50**, 74 (1957)
67. Jasper, H., Ricci, F., and Doane, B., in *A Ciba Foundation Symposium: Neurological basis of behavior*, 277-94 (J. S. A. Churchill, Ltd., London, Engl., 1958)
68. Strumwasser, F., *Science*, **127**, 469-70 (1958)
69. Rowland, V., *Electroencephal. and Clin. Neurophysiol.*, **9**, 585-94 (1957)
70. Yoshii, N., Pruvot, P., and Gastaut, H., *Electroencephal. and Clin. Neurophysiol.*, **9**, 595-608 (1957)
71. Morrell, F., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 545-60 (Little, Brown & Co., Boston, Mass., 1958)
72. Morrell, F., in *Central Nervous System and Behavior* (Brazier, M. A. B., Ed., Josiah Macy, Jr. Foundation, New York, N. Y., in press)
73. Lesse, H., *Federation Proc.*, **16**, 79 (1957)
74. Hernandez-Peón, R., Scherrer, H., and Jouvét, M., *Science*, **123**, 331 (1956)
75. Hernandez-Peón, R., Scherrer, H., and Velasco, M., *Acta neurol. Latin.*, **2**, 8 (1956)
76. Chow, K. L., Dement, W. C., and John, E. R., *J. Neurophysiol.*, **20**, 482-93 (1957)
77. John, E. R., and Killam, K. F., *J. Pharmacol. Exptl. Therap.*, **122**, 35A-37A (1958); John, E. R. in *Central nervous system and behavior* (Brazier, M. A. B., Ed., Josiah Macy, Jr., Foundation, New York, N. Y., in press)
78. John, E. R., and Killam, K. F. (Personal communication)
79. Gelber, B., *J. Comp. and Physiol. Psychol.*, **51**, 110-15 (1958)
80. Gelber, B., *Science*, **126**, 1340 (1957)

81. Jensen, D. D., *Science*, **125**, 191 (1957)
82. Jensen, D. D., *Science*, **126**, 134 (1957)
83. Mirsky, A. F., and Katz, M. S., *Science*, **127**, 1498-99 (1958)
84. Thorpe, W. H., *Learning and Instinct in Animals*, Chap. IV (Harvard University Press, Cambridge, Mass., 493 pp., 1956)
85. Loucks, R. B., *J. Psychol.*, **1**, 5-44 (1935)
86. Doty, R. W., and Giurgea, C., *The Physiologist*, **1** (Abstract, publication in August, 1958)
87. Giurgea, C., *Ber. ges. Physiol. u. exp. Pharmacol.*, **175**, 80 (Abstract, 1955)
88. Giurgea, C., *Elaborarea reflexului condiționat prin excitarea directă a scoartei cerebrale* (Editura Academiei Republicii Populare Romane, Bucharest, Romania, 153 pp., 1953)
89. Hess, E. H., *Science*, **198**, 81-93 (1958)
90. Jaynes, J., *J. Comp. and Physiol. Psychol.*, **51**, 238-42 (1958)
91. Jaynes, J., *J. Comp. and Physiol. Psychol.*, **51**, 234-37 (1958)
92. John, E. R., Wenzel, B. M., and Tschirgi, R. D., *Science*, **127**, 25-26 (1958)
93. Magoun, H. W., *Physiol. Revs.*, **30**, 455 (1950)
94. Jasper, H. H., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 319-31 (Little, Brown & Co., Boston, Mass., 1958)
95. Hugelin, A., and Bonvallet, M. J., *J. physiol. (Paris)*, **49**, 1171-1200 (1957)
96. Hugelin, A., and Bonvallet, M. J., *J. physiol. (Paris)*, **49**, 1201-23 (1957)
97. Hugelin, A., and Bonvallet, M. J., *J. physiol. (Paris)*, **49**, 1225-34 (1957)
98. Livingston, R. B., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 177-85 (Little, Brown & Co., Boston, Mass., 1958)
99. Li, C. L., and Jasper, H. H., *J. Physiol. (London)*, **121**, 117 (1953)
100. Li, C. L., Cullen, C., and Jasper, H. H., *J. Neurophysiol.*, **19**, 111 (1956)
101. Li, C. L., *J. Physiol. (London)*, **131**, 115 (1956)
102. Li, C. L., *J. Physiol. (London)*, **133**, 40 (1956)
103. Jung, R., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 423-34 (Little, Brown & Co., Boston, Mass., 1958)
104. Arduini, A., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 333-51 (Little, Brown & Co., Boston, Mass., 1958)
105. French, J. D., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 491-505 (Little, Brown & Co., Boston, Mass., 1958)
106. Scheibel, M., Scheibel, A., Mollica, A., and Moruzzi, G., *J. Neurophysiol.*, **18**, 309-31 (1955)
107. Amassian, V. E., and Waller, H. J., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 69-108 (Little, Brown & Co., Boston, Mass., 1958)
108. DeValois, R. L., Smith, C. J., Kitai, S. T., and Karoly, A. J., *Science*, **127**, 238-39 (1958)
109. Fuster, J. M., *Science*, **127**, 150 (1958)
110. French, J. D., *J. Neurosurg.*, **15**, 97-115 (1958)
111. Akimoto, H., Yamaguchi, N., Okabe, K., Nakagawa, I., Abe, K., Torii, H., and Masahashi, K., *Folia. Psychiat. et Neurol. Japon.*, **10**, 117 (1956)
112. Grundfest, H., Discussion after paper, by Li, C.-L., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 473-87 (Little, Brown & Co., Boston, Mass., 1958)
113. Arduini, A., Mancía, M., and Mechelse, K., *Riv. arch. ital. biol.*, **95** (1957, in press)

114. Brookhart, J. M., Arduini, A., Mancina, M., and Moruzzi, G., Cited by Arduini, A., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 349 (Little, Brown & Co., Boston, Mass., 1958)
115. Freeman, W. J., *Science*, **126**, 1343-44 (1957)
116. Purpura, D. P., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 435-87 (Little, Brown & Co., Boston, Mass., 1958)
117. Lindsley, D. B., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 513-34 (Little, Brown & Co., Boston, Mass., 1958)
118. Dell, P. C., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 365-79 (Little, Brown & Co., Boston, Mass., 1958)
119. Ingvar, D. H., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 381-408 (Little, Brown & Co., Boston, Mass., 1958)
120. Rothballer, A. B., *Electroencephal. and Clin. Neurophysiol.*, **9**, 409-17 (1957)
121. Purpura, D. P., and Grundfest, H., *J. Neurophysiol.*, **20**, 496-522 (1957)
122. Schlag, J., *Science*, **127**, 1184-85 (1958)
123. Osmond, H., and Smythies, J. R., *J. Mental Sci.*, **98**, 309-15 (1952)
124. Szatmari, A., Hoffer, A., and Schneider, R., *Am. J. Psychiat.*, **111**, 603-16 (1955)
125. Hoagland, H., *Ann. N. Y. Acad. Sci.*, **66**, 445-58 (1958)
126. Woolley, D. W., and Shaw, E. N., *Ann. N. Y. Acad. Sci.*, **66**, 649-67 (1957)
127. Gaddum, J. H., *Ann. N. Y. Acad. Sci.*, **66**, 643-48 (1957)
128. Brodie, B. B., and Shore, P. A., *Ann. N. Y. Acad. Sci.*, **66**, 631-42 (1957)
129. Page, I. H., *Ann. N. Y. Acad. Sci.*, **66**, 592-601 (1957)
130. Olds, J., Killam, K. F., and Eiduson, S., in *Psychotropic Drugs*, 335-43 (Garattini, S. and Ghetti, V., Eds., Elsevier Pub. Co., New York, N. Y., 1957)
131. Rothlin, E., *Ann. N. Y. Acad. Sci.*, **66**, 668-76 (1957)
132. Sigg, E. B., and Schneider, J. A., *Electroencephal. and Clin. Neurophysiol.*, **9**, 419-26 (1957)
133. Shore, P. A., and Brodie, B. B., in *Psychotropic Drugs*, 423-27 (Garattini, S. and Ghetti, V., Eds., Elsevier Pub. Co., New York, N. Y., 1957)
134. Woolley, D. W., and Edelman, P. M., *Science*, **127**, 281-82 (1958)
135. Udenfriend, S., Weissbach, H., and Bogdanski, D. F., *Ann. N. Y. Acad. Sci.*, **66**, 602-8 (1957)
136. Hess, W. R., in *The Functional Organization of the Diencephalon* (Hughes, J. R., Ed., Grune & Stratton, Inc., New York, N. Y., 180 pp., 1957)
137. Carlsson, A., Rosengren, E., Bertler, A., and Nilsson, J., in *Psychotropic Drugs*, 363-72 (Garattini, S. and Ghetti, V., Eds., Elsevier Pub. Co., New York, N. Y., 1957)
138. Shore, P. A., and Brodie, B. B., *Science*, **127**, 704 (1958)
139. Vogt, M., *Brit. Med. Bull.*, **13**, 166-71 (1957)
140. Travis, R. P., and Olds, J. (Unpublished data)
141. Zimbardo, P. G., and Barry, H., III, *Science*, **127**, 84-85 (1958)
142. Olds, J., and Olds, M. E., *Science*, **127**, 1175-76 (1958)
143. Elkes, J., in *Neuropharmacology*, 205 (Abrahamson, H. A., Ed., Josiah Macy, Jr. Foundation, New York, N. Y., 1957)
144. Bradley, P. B., in *Henry Ford Hospital International Symposium: The Reticular Formation of the Brain*, 123-49 (Little, Brown & Co., Boston, Mass., 1958)
145. Olds, J., Travis, R. P., and Schwing, R. (Unpublished data)
146. Olds, J., *J. Comp. and Physiol. Psychol.*, **52** (In press, 1959)

ADENOHYPOPHYSIS AND ADRENAL CORTEX¹

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The pace and the emphasis have changed, in recent years, in work on the pituitary and adrenal cortex. The excitement of the 1949-1950 discoveries of new therapeutic uses of corticotropin and cortisone has subsided, and in its place one finds a new focus of attention on the control of the function of the adenohypophysis. Newer methods of separating and identifying small amounts of steroids have facilitated the study of the biosynthesis and catabolism of the adrenocortical hormones. The recent discovery of aldosterone has stimulated anew work on the adrenal involvement in the control of salt and water metabolism.

ADENOHYPOPHYSIS

LOCALIZATION OF HORMONES

The localization of the gonadotropes and thyrotropes has been fairly clearly established, and the source of growth hormone is suggested by pathological findings in dwarfism and gigantism. Involvement of the basophils in production of corticotropin was suggested by nuclear and nucleolar hypertrophy, associated with some cytoplasmic degranulation, in pituitary basophils of patients treated with cortisone and corticotropin [Montandon (1)]. The highest concentration of corticotropin in beef, hog, and human pituitaries was found in an area rich in basophilic cells, lying in the antero-medial portion of the anterior lobe [Rochefort & Saffran (2)]. In the rat the distribution of both the hormone and the basophils is more uniform.

Changes in corticotropin secretion are not accompanied by the profound signs of granulation and degranulation that are seen in other secreting cells. This lack led to the suggestion that the chromophobic cells of the adenohypophysis are responsible for corticotropin secretion. Another possibility was suggested by Farquhar's (3) discovery, using the electron microscope, of small follicles, which cannot be distinguished by light microscopy. These are larger under conditions of corticotropin storage (after cortisone), and smaller, with corticotropin depletion (uniadrenalectomy). The material in the follicles may represent stored corticotropin. The relative lack of formed elements in the cells surrounding the follicles may explain the difficulty in relating secretion with changes in granulation. This system is analogous in some ways to the thyroid.

BIOLOGICAL ASSAY

The protein or peptide nature and the minute amounts of the adenohypophysial hormones offer little scope for the development of chemical

¹ The survey of the literature on which this review is based was concluded in May, 1958.

methods of determination. These facts necessitate the use of biological assays, with all their inherent faults of relative lack of speed and precision and with difficulty of performance. But, bioassays are often endowed with extreme sensitivity, far beyond the limits of chemical methods, and with relative specificity. A recent symposium has dealt with the problems associated with the measurement of hormones in blood (4), and Loraine (5) has written a useful volume entitled *Clinical Application of Hormone Assay*, which deals with chemical as well as biological assay.

Corticotropin.—No radically new methods have been proposed for the bioassay of corticotropin. The increase in plasma "hydrocortisone" after the intravenous administration of corticotropin to man has been suggested as a basis for an assay [Persky & Heath (6)]. The relatively satisfactory precision is offset by the inconvenience of intravenous administration of each test dose over a period of 24 hours and by the difficulties associated with measurements of corticoids in plasma. The *in vitro* assay of corticotropin and the classical ascorbic acid depleting method were compared in the same laboratory for the first time by Van der Vies (7).

Loraine (8) has warned of the dangers of applying bioassays to untreated biological materials, which may contain potentiating or depressing agents. Serum of normal or scorbutic guinea pigs has the property of increasing the slope of the dose-response curve to corticotropin in the adrenal ascorbic acid-depleting method (9), while rat serum proteins, even from hypophysectomized animals, increased the production of corticoids by rat adrenals in the *in vitro* assay (10). Even samples treated to concentrate corticotropin may contain interfering material. Fresh human placentae, extracted with acetic acid, and the extracts treated with oxycellulose by procedures used to concentrate corticotropin, yielded a material with ascorbic acid-depleting activity in hypophysectomized rats; a significant amount of activity was retained after boiling at pH 9 or 12 (11).

Gonadotropin.—The early action of gonadotropin is to promote the secretion of a uterine-weight stimulating material by the ovary. This activity was displayed by all gonadotropin preparations tested so far, including human pregnancy urine gonadotropin, human postmenopausal urine gonadotropin, pregnant mare serum, hog pituitary FSH, sheep pituitary LH and a purified sheep anterior pituitary preparation [Emmens, Claringbold & Lamond (12, 13, 14)]. Existing methods were used to assay a variety of preparations of gonadotropin. No one assay was suitable for all preparations [Apostolakis & Voigt (15)]. Rosenberg, Smith & Dorfman (16) assumed that the ovarian uterotrophic agent is estrogen and have proposed an assay for gonadotropin using estrone as a standard. The application of this principle of using a target hormone as a standard for the assay of pituitary tropic hormones could be extended to other systems in which a pure standard is not available. Pugsley (17) has applied the principle of the cessation of estrus cycles in adult rats by a multivitamin deficiency to provide convenient test animals which can be used repeatedly.

The depletion of ovarian ascorbic acid has been adapted to the bioassay

of gonadotropin [Parlow (18)]. The method seems to be relatively specific for LH. Noach & van Rees (19) also observed the depletion of ovarian ascorbic acid after the administration of gonadotropin to immature rats, but concluded that an assay based on this phenomenon would have no advantages over existing methods. Segal (20) has observed that regenerating feathers in a bird, the weaver finch, displayed a black bar after treatment with as little as 5 μ g. of LH.

Thyrotropin.—Adams & Purves (21) have designed an assay based upon the increase in plasma ^{131}I in guinea pigs pretreated with ^{131}I . A novel principle is employed in an *in vitro* assay for thyrotropin: beef thyroid slices swell in the presence of thyrotropin, and the increase in weight is proportional to the log dose (22). This effect of thyrotropin may be exerted by an acceleration of the breakdown of storage forms of thyroid protein into smaller molecules, the consequent increase in colloid osmotic pressure resulting in imbibition of water and swelling.

PREPARATION AND PROPERTIES

Gonadotropins.—Modern methods of separation have been applied to the purification of gonadotropins. Li and his colleagues (23) have used salt and solvent partitions, chromatography, and electrophoresis to prepare from sheep pituitaries LH which was active at a total dose of 2 μ g., and which was free of significant amounts of other adeno-hypophysial hormones, including FSH. They also concentrated the gonadotropic material from pregnant mare serum but failed to separate the FSH and LH activities (24). The ratio of activities remained constant during purification and, contrary to an earlier report, exhibited the same sensitivity to periodate. The authors suggested that the two activities are inherent properties of the same molecule, the predominant effect depending upon the dose and the assay system. Buchholz (25) found a ratio of unity throughout the menstrual cycle between total urinary gonadotropin and LH, as measured by its effect on the prostate of hypophysectomized rats. Others [Butt and coworkers (26); Fletcher & Brown (27); Walter (28); Hamburger & Johnsen (29)] have attempted to separate the chorionic gonadotropins in human urine. The evidence for separate entities is suggestive, but far from conclusive. The apparent dual role of the gonadotropins in blood and urine is in contrast to the relatively clear separation of biological activity between hypophysial FSH and LH. Rigid purification and chemical characterization of these hormones in blood and urine are required to establish their relationship.

Gonadotropic activity is associated with pituitary preparations containing large amounts of bound carbohydrate (30), while galactosamine has been identified in human chorionic gonadotropin (31). Bourrillon *et al.* (32) have suggested that an α_1 -mucoprotein serves as a carrier for gonadotropin in pregnant mare serum. The latter gonadotropin can be split into a biologically inert mucoprotein and an active fraction with enhanced potency.

There is evidence for species specificity of gonadotropin. Multiple ovulation occurred consistently in monkeys given primate gonadotropin, in con-

trast to sporadic responses to sheep preparations [van Wangenen & Simpson (33)]. The immature chicken responded normally only to chicken pituitary powder; mammalian gonadotropin preparations caused cystic ovaries [Taber *et al.* (34)]. In addition, gonadotropins of various species displayed immunologic differences [Henry & van Dyke (35); Cole, Hamburger & Neimann-Sørensen (36)]. Gonadotropic activity in pooled human plasma was concentrated in fraction III-0, which contains the β -lipoproteins and albumin [Antoniades *et al.* (37)].

Prolactin, from sheep pituitaries, is a protein with a molecular weight of 24,200 according to ultracentrifugation, end-group analysis, and amino acid composition (38). Performic acid oxidation of the —S—S— link in prolactin did not result in the formation of peptide fragments, suggesting that another ring structure is present in the molecule in addition to a poly —S—S— bridge (39). The mammatropic activities in blood and urine of women have been ascribed to prolactin on the basis of the action on mammary gland growth in male mice primed with estrogen and progesterone (40, 41). A curious property of prolactin is its ability to promote melanogenesis in already-present melanophores of hypophysectomized killifish (*Fundulus*), while intermedin causes proliferation of melanophores (42).

Thyrotropin.—Bates (43) has described a startlingly simple method of preparing thyrotropin by percolating ethanol with increasing amounts of water through pituitary powders. The same principle was applied to the extraction of thyrotropin from lyophilized human plasma, which contains the equivalent of about 50 milliunits per 100 ml. The pepsin-inhibitor, previously reported to be a property of thyrotropin preparations, has been separated from the hormone by countercurrent distribution (44) and by simple solvent partition (45).

Chemical alteration of the thyrotropin molecule by acetylation or sulfation not only caused loss of hormonal activity, but the acetylated hormone also competed with the native hormone for binding sites on the thyroid, contrary to the finding with gonadotropin [Sonenberg & Money (46)]. The exophthalmic activity in thyrotropin persisted after the action on the thyroid was destroyed by peptic digestion; tryptic digestion abolished all activities [Brunish (47)].

Growth hormone.—The species specificity of growth hormone has stimulated its isolation from a variety of species. Raben (48), Li (49), and Gemzell & Li (50) have prepared human and monkey growth hormone. The human pituitary gland contains about the same amount of growth hormone activity as glands from cattle, sheep, and monkeys. Although both human and monkey growth hormone are able to produce metabolic signs of growth in man (51), these two preparations are chemically different (49). The rhesus monkey responded to the monkey hormone, but not to bovine or porcine preparations [Knobil *et al.* (52)]. Human growth hormone failed to stimulate the formation of precipitating antibodies in rodents (53). Papkoff & Li (54) described the isolation of the hormone from whale pituitaries and found it to differ chemically from that of other species. It was active in the rat tibia test.

Corticotropin.—The biological activity of corticotropin is decreased by oxidation with peroxide and can be restored by cysteine and other reducing thiols (55). The loss in activity could not be attributed to any change in the amino acids of the corticotropin molecule, suggesting that an as-yet-unidentified group may be present to account for the lability of the hormone.

Solem, Holtermann & Skogrand (56) found that corticotropin prepared from infantile human pituitary glands was no more effective than commercial preparations in promoting an increased excretion of 17-ketosteroids. Thus, the relatively high 17-ketosteroid excretion in infants is caused by a difference at the adrenal level rather than by the presence of a specific 17-ketosteroid tropic factor in the infantile pituitary.

Sebotropic activity.—Loringz & Lancaster (57) described the preparation of an extract of anterior lobe that, given to hypophysectomized rats along with progesterone, caused an increase in the size of the preputial, Harderian, and cutaneous sebaceous glands. They could not find similar activity in the well-characterized anterior lobe hormones.

CONTROL OF ANTERIOR PITUITARY SECRETION

Corticotropin.—The current interest in the regulation of the secretion of corticotropin is illustrated by the comprehensive review of the subject by Sayers in *Annual Review of Physiology* for 1958 (58). At first glance the problem seems only to require the isolation and identification of an hypothalamic corticotropin-releasing factor for which the apt name adrenopituitropic hormone has been suggested (59). Numerous candidates have been proposed: a lipide or lipoprotein, by Slusher & Roberts (60); a protein, by Porter & Rumsfeld (61); vasopressin, by Martini & Morpurgo (62), Sobel and co-workers (63), McCann (64), and others; a distinct neurohypophysial peptide, by Guillemin and his group (65) and by Saffran, Schally & Benfey (66); and histamine, by Fuche & Kahlson (67). Each substance has a good claim to the title of corticotropin-releasing factor, but conclusive evidence for the physiological role of any is lacking.

Vasopressin, the earliest suggested corticotropin-releasing factor, is still receiving strong support. McCann & Fruit (68), using rats bearing hypothalamic lesions, found that adrenal ascorbic acid depletion was proportional to the pressor activity in commercial and synthetic vasopressin, suggesting that the effect on the adrenal was caused by the vasopressin, and not by a contaminant in the commercial preparation. Paper chromatography of commercial vasopressin failed to separate a nonpressor corticotropin-releasing fraction [McCann (64)]. However, corticotropin-release by vasopressin was observed only with relatively high doses. A maximum depletion of adrenal ascorbic acid is usually seen with as little as five milliunits of corticotropin, whereas five units of vasopressin are required to produce as great a depletion. This dose is about 1000 times the dose that causes maximal anti-diuresis, implying a very great difference in the sensitivity of the kidney and the adenohypophysis to vasopressin. Nevertheless, Casentini, de Poli & Martini (69) suggested that the simultaneous antidiuresis and adrenal-

stimulating effects of acetylcholine in intact rats are mediated by vasopressin. Leeman & Munson (70) have tested vasopressin in a pharmacologic system designed to detect the corticotropin-releasing factor and obtained suggestive, but nevertheless equivocal, evidence for its activity.

In 1951 Nagareda & Gaunt (71) observed that a water load in rats caused signs of adrenocortical stimulation, although the release of vasopressin was clearly inhibited because the water was promptly excreted. This observation demonstrated a dissociation between the release of corticotropin and of vasopressin. Similar experiments, both in man (72) and with guinea pigs (73) have caused former adherents to the theory of corticotropin-release by vasopressin to doubt the role of vasopressin in the pituitary-adrenal response to stress. Further objections to vasopressin have been raised by Royce & Sayers (58, 74), who pointed out that vasopressin may apparently release corticotropin by the following nonspecific actions: (a) stimulation of the central nervous system, (b) a direct adrenal ascorbic acid depleting activity, manifested in hypophysectomized rats, (c) an increase in circulating "corticotropin" in hypophysectomized or decapitated rats by liberation from extra-pituitary stores, or (d) inhibition of the destruction of corticotropin.

The interesting proposal of Slusher & Roberts (60), that the corticotropin-releasing factor is a lipid or lipoprotein material obtained from the posterior hypothalamus, has been reexamined by de Wied (75, 76). Such extracts depleted adrenal ascorbic acid in intact, but not in hypophysectomized rats. However, the lipid material was inactive in rats treated with chlorpromazine or morphine, suggesting an action via the central nervous system rather than directly on the pituitary. Hypothalamic lipid extracts were reported by Curri & Paoletti (77) to promote the growth of rats in a manner similar to growth hormone.

A neurohypophysial peptide corticotropin-releasing factor, distinct from vasopressin, has been tested in man (78). A dose of 5 to 10 mg. intravenously of relatively impure material caused a prolonged increase in blood and urinary corticoids, whereas 25 to 45 μ g. of a purified fraction resulted in a transient elevation of blood corticoids, with a maximum at 15 min. A similar, or perhaps identical, peptide has been purified from posterior pituitary extracts by Schally, Saffran & Zimmermann (79). This material significantly stimulated the release of corticotropin from rat anterior pituitary tissue *in vitro* at a dose of 1 μ g. or less. An amino acid analysis revealed a similar composition to that of vasopressin, except that serine and histidine, which are not present in oxytocin and vasopressin, were always found in active corticotropin-releasing preparations. Like Guillemin's material, this substance was relatively free of pressor and oxytocic activity, but did possess significant antidiuretic potency (80). In microgram doses, it caused a 50 per cent decrease in the pituitary stores of an intact anesthetized rat, whereas similar doses of vasopressin had little, if any, effect (81). It also stimulated the incorporation of 32 P into phospholipides of anterior pituitary tissue, a phenomenon that has been found to accompany the activation of the release of stored peptides by specific stimulants [Hokin *et al.* (82)].

Vasopressin, oxytocin, and the corticotropin-releasing factor of Schally *et al.* are chemically similar and it is not surprising that they share, to some extent, each other's major biological activities. Just as vasopressin has weak but definite oxytocic properties, vasopressin possesses some corticotropin-releasing activity. This would explain the finding of McCann and others that vasopressin releases corticotropin, but only at relatively elevated doses.

One of the objections to the participation of a neurohypophysial factor in the pituitary-adrenal response to stress has been the difficulty in explaining the transport of the factor to the adenohypophysis. In 1951 Landsmeer (83) described extensive anastomoses between the vessels of the two lobes of the rat hypophysis, and more recently Jewell (84) reported that similar connections are present in the dog.

Fortier & Ward (85, 86) have questioned the specificity of the *in vitro* test for corticotropin-releasing activity used by Saffran and Guillemin and their co-workers. Relatively large doses of serum extracts apparently stimulated the release of corticotropin from isolated rat anterior pituitary tissue, and the activity of the extracts was not influenced by prior treatment of the donor animals (stressed or not). They suggested that the extracts protected the corticotropin released from the tissue against destruction rather than stimulating the release, and advised caution in interpreting results based upon the *in vitro* test. Fortier & Ward assayed the corticotropin by an *in vitro* method, which itself has been criticized as lacking specificity in the presence of blood extracts (10). However, the amount of material in the extracts was far in excess of the effective doses of corticotropin-releasing factor employed by the originators of the test.

Barrett & Sayers (87) also doubted the validity of the *in vitro* test for corticotropin-releasing activity. Like Fortier, they believed that apparent increased release of corticotropin from isolated rat pituitaries resulted from protection of corticotropin by basic peptides. Evidence in favor of this interpretation was obtained in experiments in which the corticotropin in whole rat pituitary glands, incubated for 8 hr., was found to be significantly decreased, and in experiments with homogenized rat pituitary tissue, in which the corticotropin was almost entirely inactivated in an hour. In both cases, basic peptides such as commercial vasopressin or glucagon, protected the corticotropin. However, there was no detectable inactivation of corticotropin in whole rat pituitaries in an incubation of $\frac{1}{2}$ hr. In these experiments corticotropin was assayed by the ascorbic acid depletion method.

In rebuttal, Saffran (88) pointed out that the *in vitro* test for corticotropin releasing activity is conducted with halved rat pituitary glands incubated for 1 hr., conditions which are more similar to those in which Barrett and Sayers could not find inactivation than to the 8-hr. incubation period or to the use of homogenized tissue. Moreover, the *in vitro* assay for corticotropin did not reveal any significant inactivation under the conditions used in the *in vitro* test for corticotropin-releasing activity [Birmingham & Kurlents (89)].

Histamine has been intermittently suggested as the corticotropin-releas-

ing factor, but usually excessively large doses were employed. However, relatively minute amounts (10 $\mu\text{g./kg.}$) of histamine were able to cause signs of adrenocortical stimulation in normal, but not in hypophysectomized rabbits (67). The low dose suggested to the authors that histamine might be an intermediate in the pituitary-adrenal response to stress, with a possible site of action on central nervous centers, rather than on the adeno-hypophysis.

Even though the identity of the corticotropin-releasing factor(s) may be uncertain, there is agreement that the response to stress is mediated by the central nervous system. The development of the ability of the neonatal rat to respond to stress parallels the development of the nervous system [Eskine (90)]. Progressive removal of the brain of the dog failed to prevent the adrenal response to operative trauma until the hypothalamus was reached [Egdahl Story & Melby (91)]. Removal of the hypothalamus abolished the adrenal response. Schmid *et al.* (92) explored carefully the hypothalamic area of the guinea pig with a coagulating electrode and located a zone in the middle of the hypothalamus that was essential for the pituitary-adrenal response to diphtheria toxin. In the rat, the location of the zone is not known with certainty, but there are indications that an area in the anterior hypothalamus is involved in the release of corticotropin [Bouman, Gaarenstroom, Smelik & de Wied (93)].

Transection of the midbrain in dogs abolished the release of corticotropin in response to stress [Anderson *et al.* (94)] and patients with complete transverse lesions of the spinal cord above T.5 exhibited significantly lower excretion of 17-ketogenic steroids than either normal subjects or patients with lesions below T.5 [Robinson (95)].

The localization of the essential neural structures in the hypothalamus and below suggests strongly that the substances which inhibit the response to stress must act on these regions. Chlorpromazine has been extensively studied, as an example of a tranquilizer, and it has been found to prevent the depletion of adrenal ascorbic acid after a variety of stresses (96). Contrary to others, this report claimed that chlorpromazine is itself not an important stressing agent. In man, as in other species, morphine blocked the response to stress (97).

The adrenal response to stress is also suppressed by adrenocortical steroids, but their site of action is not clear. The bulk of the evidence suggests that the steroids act on a central nervous structure to prevent the secretion of the corticotropin-releasing factor(s) (98). However, there are hints that the adrenal cortex itself may be influenced by high concentrations of its own products. In hypophysectomized rats, the sensitivity of the adrenals to corticotropin was found to be decreased when the animals were treated with cortisone [Langecker & Lurie (99)]. The unstimulated production of corticoids by rat adrenals *in vitro* is decidedly suppressed by the addition of adrenal steroids to the incubation medium [Birmingham & Kurlents (89)]. The significance of the direct effect of adrenal steroids on the adrenal cortex has not yet been fully evaluated, but it emphasizes the need for caution in the interpretation of experiments using massive doses of steroids to block the response to stress.

The neurohypophysis contains substantial amounts of corticotropin (2, 100) and this corticotropin was depleted by sound, whereas the adeno-hypophyseal corticotropin was depleted by histamine (100). Direct neural control of the adeno-hypophyseal release of corticotropin is apparently ruled out by the sparse nerve supply to the organ, but the neurohypophysis is amply supplied with nervous connections with the hypothalamus.

The direct effect of circulating corticotropin on adeno-hypophyseal stores was investigated by Kitay, Holub & Jailer (101). In normal and in adrenalectomized rats, the administration of corticotropin resulted in a doubling of the pituitary content. In contrast, cortisone caused a decrease. An ingenious method for determining the rate of discharge of corticotropin was described by Long & Bonnycastle (102). The potential importance of this procedure in the search for modifiers of corticotropin secretion is very great.

Gonadotropins.—Less attention has been paid to the study of the control of the release of the gonadotropins than of corticotropin. However, there are marked similarities apparent in the mechanisms involved. An hypothalamic neurohumor, transported by a direct vascular connection with the adeno-hypophysis, is postulated to effect the discharge of the gonadotropins. The importance of the proximity of the pituitary to the hypothalamus was again demonstrated, this time in the frog, by the inability of pituitary transplant in the oculomotor muscle to maintain gonadal activity [Vivien & Schott (103)]. In rats, pituitary transplants in the eye or on the kidney were able to secrete prolactin, as indicated by a decidual reaction in response to uterine trauma, regardless of the time of the cycle when the pituitary was transplanted [Nikitovitch-Winer & Everett (104)]. Bilateral lesions near the ventromedial nucleus of the rat hypothalamus nearly always caused persistent cornification of the vaginal epithelium (105), while destruction of the arcuate nucleus led to signs of gonadal underfunction (106). In the cat, electrical stimulation of the mammillary body or of the ventromedial nucleus caused ovulation, while destruction of these areas prevented ovulation [Robison & Sawyer (107)].

The selectivity of hypothalamic lesions has been questioned by Herbert & Zuckerman (108), who observed that insertion of coagulating electrodes, without passing a current, into any part of the brain of the ferret produced the same effect on the estrus cycle as lesions in the anterior hypothalamus. Chlorpromazine and reserpine, which act on the central nervous system, prevented ovulation if administered to the rat before the "critical time" on the day of ovulation [Barraclough & Sawyer (109)]. Gitsch & Everett (110) found that yet another anticholinergic drug blocked ovulation in the rat if given at the proper time. Adrenolytic agents, such as *N*-(2-chloroethyl)-dibenzylamine (Dibenamine) and phenoxybenzamine (Dibenzylamine), have been long known to behave similarly and this has been interpreted as evidence for the participation of a sympathetic step in the control of ovulation. However, if these agents are administered chronically, spontaneous ovulation returns [Moore (111)]. This adaptation was interpreted as support for the hypothesis that sympathetic blockaders "prevent the release of ovulating hormone indirectly by producing a shift in the normal secretion by the

adenohypophysis to ACTH". The apparent reciprocal relationship between corticotropin and gonadotropin has been considered repeatedly. Further support was furnished for this idea by the work of Nowell & Chester Jones (112), who found that the storage of corticotropin in the pituitary gland seems to be diminished when the storage of gonadotropin is high.

The identity of the hypothalamic gonadotropin-releasing factor(s) is still obscure. Oxytocin was observed to shorten materially the bovine estrus cycle [Armstrong & Hansel (113)]. On the other hand, commercial vasopressin and epinephrine, but not oxytocin, were able to cause a depletion of ovarian ascorbic acid, which indicated a release of LH (18). The apparent role of oxytocin in the release of prolactin has been supported by the work of McCann, Mack & Gale (114) on the persistence of mammary gland development after weaning by the administration of oxytocin. Reserpine also stimulated mammary growth and lactation, suggesting that it promoted the synthesis of prolactin [Meites (115)]. In fact, reserpine increased the amount of prolactin in the pituitary gland. The intervention, if any, of oxytocin in the mammary response to reserpine has not been explored.

The suppression of gonadotropin release by the sex hormones has long been known. New evidence indicates that estrogens exert this effect by direct action on the brain. Fragments of ovarian tissue, grafted near the paraventricular nuclei, caused a decrease in uterine weight of otherwise normal female rats [Flerkó & Szentágothai (116)]. In five of six menopausal women, the administration of progesterone led to a decreased excretion of gonadotropin [Rothchild (117)]. Testosterone propionate caused ovarian atrophy in rats; this effect was overcome by the administration of chorionic gonadotropin [Gans & de Jongh (118)]. In nonvirilizing doses, a synthetic anabolic steroid, norethandrolone, suppressed the secretion of gonadotropin [Goldman, Epstein & Kupperman (119)]. Cortisone, on the other hand, had no effect on the excretion of gonadotropin in a castrate woman [Smith & Albert (120)].

Thyrotropin.—The release of thyrotropin is also under the control of the nervous system. Lesions in the hypothalamus resulted in signs of thyroid understimulation [Bogdanove & Bogdanove (121); D'Angelo (122)], but the pituitary stores of thyrotropin were essentially normal (122), indicating the autonomy of the thyrotropin-synthesizing system. Lesions in the median eminence, or the administration of reserpine, did not block the release of ^{131}I from the thyroid, but did prevent the increased release in response to cold [Knigge & Bierman (123)]. Morphine also suppressed the release of thyrotropin [Samel (124)]. There is no direct evidence for the existence of a thyrotropin releasing factor(s). Hypothalamic tissue added to mouse pituitaries in tissue culture failed to renew the secretion of thyrotropin (125).

ADRENAL CORTEX

ANABOLISM OF ADRENOCORTICAL STEROIDS

Cholesterol.—Rapid progress has been made in determining the pathway of biosynthesis of adrenocortical hormones [reviewed by Dorfman (126)]

and by Pincus (127)]. Isotopic methods, in particular, have led to the understanding of the intermediates involved in the condensation of acetate molecules to form cholesterol, which is believed to be the first steroid substance formed in the biosynthesis of the adrenal hormones [Bloch (128)]. The long, 30-carbon molecule, squalene, is formed from acetate and cyclized into cholesterol. Recently, the 6-carbon compound, mevalonic acid, or β -methyl- β , Δ -dihydroxyvaleric acid, was found to be an intermediate in the building-up of acetate to squalene [Wright & Cleland (129); Dituri *et al.* (130)] (Figure 1, steps I and II).

Direct proof for the conversion of cholesterol to adrenocortical hormones was provided by studies in which ^3H or ^{14}C -labelled cholesterol was utilized by the adrenal cortex *in vitro* [Kurath, Ganis & Radakovich (131)] and *in vivo* [Werbin, Chaikoff & Jones (132)] to form isotopically-labelled cortisol. Moreover, the feeding of Δ^4 -cholestenone, which inhibits cholesterol synthesis, resulted in signs of adrenocortical underfunction, including an increased adrenal weight and profound decreases in plasma levels of corticosterone and aldosterone [Fredrickson, Peterson & Steinberg (133)]. But the status of cholesterol as an obligatory intermediate was seriously challenged by the observation that ^{14}C -acetate could be introduced into cortisol and corticosterone by adrenal homogenates without detectable labelling of the cholesterol [Webb & Heard (134)]. This work adds to the evidence for the existence of an alternate pathway for the formation of the adrenocortical hormones from acetate (Step XV).

Role of progesterone.—According to Figure 1, progesterone occupies a key position in the formation of the adrenocortical hormones. Progesterone- $4\text{-}^{14}\text{C}$, added to minced human adrenal tissue, was converted to 11-deoxycorticosterone (Step VI), corticosterone (Step VII), 17-hydroxyprogesterone (Step XII) and cortisol (Steps XIII and XIV) [Berliner, Berliner & Dougherty (135)]. Adrenal tissue from a case of Cushing's syndrome converted the progesterone to the other compounds at a much more rapid rate, but 17-hydroxyprogesterone could not be detected. Perhaps the 17-hydroxyprogesterone was converted to cortisol as rapidly as it was formed. No 11 β -hydroxyprogesterone (Step IX) nor aldosterone (Step VIII) could be found. The adrenal mince produced more corticosterone than cortisol from progesterone, although the human gland is well known to produce cortisol as the major product. This implies that progesterone is not involved in the by-pass (Step XV) from acetate to cortisol.

Hydroxylases.—In perfused calf adrenals, $4\text{-}^{14}\text{C}$ -progesterone was found to be converted to aldosterone [Chen *et al.* (136)]. This conversion is apparently confined to the zona glomerulosa. Stachenko & Giroud (137), using the separated zones of beef adrenal glands, observed an increased formation of aldosterone after the addition of progesterone, 11 β -hydroxyprogesterone, 11-deoxycorticosterone and corticosterone to glomerulosa, but under no circumstances was aldosterone formed by the fasciculata-reticularis. On the other hand, progesterone gave rise to cortisol only with fasciculata-reticularis. Thus, the 17-hydroxylating enzyme (Step XII) is localized in the fasciculata-reticularis, while the 18-hydroxylase (Step VIII) is in the

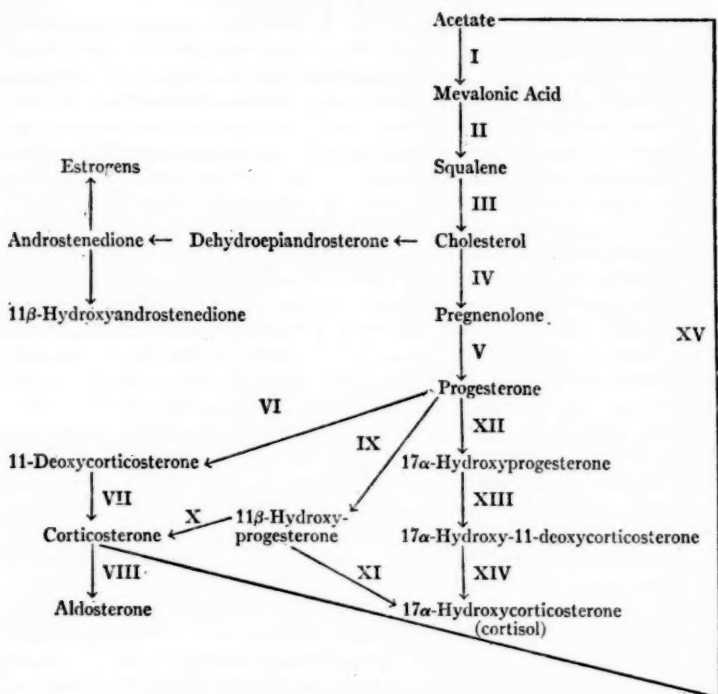


FIG. 1. Anabolism of Adrenocortical Steroids.

glomerulosa. All zones were, however, able to form cortisol from 17α -hydroxyprogesterone and 17α -hydroxy-11-deoxycorticosterone (Steps XIII and XIV), which demonstrated that the 11- and 21-hydroxylases are widely distributed in the adrenal cortex. The fact that corticosterone is convertible to aldosterone (Step VIII) may explain the minor stimulation of aldosterone production by corticotropin, which is known to increase the blood level of corticosterone, presumably produced in the fasciculata-reticularis.

In both perfused and homogenized beef adrenals, ^{14}C - 11β -hydroxyprogesterone produced only minor amounts of labelled corticosterone (Step X), but variable yields of cortisol (Step XI) [Eichhorn & Hechter (138)]. However, the authors concluded that the rapid conversion to cortisol was an artifact of homogenization in ionic media, and preferred to relegate 11β -hydroxyprogesterone to a minor role in the biosynthesis of adrenocortical hormones. The same authors [Eichhorn & Hechter (139)] cleared up another controversy by showing that with cow adrenal homogenates ^{14}C -labelled 11-deoxycorticosterone gave rise, not to cortisol, as had been claimed by others, but to another substance with similar chromatographic properties, separable from cortisol after acetylation. 11-Deoxycorticosterone formed

corticosterone (Step VII), and progesterone formed both corticosterone and cortisol. This paper explains away the apparent exception to the rule that a hydroxyl on C-21 prevents subsequent hydroxylation on C-17.

The adrenal gland is capable of introducing an 11 β -hydroxyl group into a wide variety of compounds (which explains the relatively small amounts of 11-deoxysteroids found in adrenal extracts). Ethynyl, vinyl, and ethyl-testosterone were 11 β -hydroxylated on adrenal perfusion [Marshall *et al.* (140)]. The "natural" substrates of the enzyme, 11-deoxycorticosterone and 17-hydroxy-11-deoxycorticosterone, are converted into corticosterone (Step VII) and cortisol (Step XIV) respectively. In the human gland at least, the activity of the enzyme has been reported to be increased by treatment with corticotropin [Grant, Symington & Duguid (141)]. The 11 β -hydroxylase step requires reduced triphosphopyridine nucleotide (TPNH), molecular oxygen, and several enzymes, which are present in adrenal mitochondria, as well as a heat stable cofactor [Tomkins, Curran & Michael (142)]. The reaction *in vitro* is inhibited by a large variety of ions [Tomkins & Michael (143)], but the significance of this inhibition is not clear. An elegant abstract by Hayano, Gut & Peterson (144) contains evidence that the 11-hydroxyl group replaces the hydrogen in the position that is hydroxylated. The relatively rapid 11 β -hydroxylation of 6 α -hydroxyandrostenedione compared to the 6 β -isomer also suggests that the 11 β -hydroxylase approaches the molecule from the unhindered β side in the 6 α -steroid [Meyer (145)]. The complete characterization of the 11-hydroxylase reaction is at hand, and it is safe to predict that the introduction of the other reactive groups in the adrenocortical hormones will be studied as intensively. Talalay (146) has reviewed the enzymes of steroid metabolism.

17-Ketosteroids.—The origin of the adrenal C₁₉ steroids, including some of the 17-ketosteroids, is still uncertain and presents a challenge to the investigator. In the fetus there is evidence for the production of 17-ketosteroids in the so-called fetal zone of the adrenal which persists for a few days after birth (56, 147). The gland of the anencephalic monster has no fetal zone, and 17-ketosteroids are absent from its umbilical blood [Nichols, Lescure & Migeon (148)].

Estrogens.—Estrone was among the substances first isolated from adrenocortical extracts. The amounts normally produced by the adrenal cortex are so small as to escape detection, but estrogens may be found in measurable amounts under conditions of adrenal hyperfunction [West, Damast & Pearson (149); Bush *et al.* (150)]. However, occasional cases of adrenal carcinoma exhibit feminization, which ceases after adrenalectomy. The steroids in feminizing tumors included progesterone, equilenin, and six unidentified compounds with phenolic properties [Salhanick & Berliner (151); Wallach *et al.* (152)]. These are the first demonstrations of equilenin in human material. In the urine of a young girl with such a tumor abnormally large amounts of estrone, estradiol, and estriol were found [Snaith (153)], indicating that these estrogens were also formed [cf. Wolf *et al.* (154)]. The adrenal cortex is able to form at least trace amounts of all classes of steroid hormones. Exaggerated production of one or more of the hormones

can occur in hyperplastic or neoplastic adrenals, leading to diseases as varied as aldosteronism, Cushing's syndrome, adrenogenital syndrome, virilization, and feminization. These adrenal diseases present an opportunity for the study of enzyme alterations in the pathways of steroid anabolism.

Blood corticosteroids.—The qualitative and quantitative differences in the steroids formed by the adrenal glands of the different species have their origin in the relative activities of the hydroxylating enzymes. Rodents apparently lack an active 17-hydroxylating system, accounting for corticosterone as the major adrenocortical product. The limited ability of the adrenal cortex to store steroids allows some of the intermediates to escape into the circulation, especially under conditions of hyperactivity. As a result, the blood contains a variety of steroids originating in the adrenal cortex. 17-Hydroxy-11-deoxycorticosterone has recently been identified in human adrenal venous blood, which also contains cortisol, corticosterone, and 11 β -hydroxyandrostenedione [Touchstone (155)]. Human plasma corticosterone and cortisol levels were parallel over a wide range of adrenal function [Peterson (155a)]. But balance between enzymes may be shifted within a species. For example, excised human adrenals secreted cortisol and corticosterone into adrenal vein blood in a ratio of 2.3 or less, whereas when the gland was removed after a course of treatment with corticotropin, the relative amount of cortisol was increased and the ratio was three or higher [Grant, Forrest & Symington (156)]. Corticosterone is, in part, formed in the glomerulosa, where it is presumably free of control by corticotropin, allowing only the extraglomerular corticosterone to respond. On the other hand, the total production of cortisol is increased by corticotropin, leading to an increase in the cortisol/corticosterone ratio.

In the mouse, the exposure of the adrenal to a constant stimulation by a corticotropin-producing pituitary tumor failed to change qualitatively the output of the adrenal cortex, which has as its main products equal amounts of corticosterone and 11 β -hydroxy- Δ^4 -androstene-3,17-dione [Wilson, Borris & Bahn (157)]. Functioning adrenal tumors of mice and rats also manufactured the same products as normal adrenals of the same species [Cohen, Bloch & Celozzi (158)]. Rat blood contains another important steroid, intermediate in polarity between corticosterone and cortisol [Reif & Longwell (159)]. This unidentified component has been found before, both in blood and in the incubation medium of rat adrenal glands *in vitro*. An interesting property of this material is the presence of the ultraviolet-absorbing Δ^4 -3-ketone and a positive Porter-Silber test for the dihydroxyacetone side-chain, in the almost total absence of reducing activity, whereas the true dihydroxyacetone group is strongly reducing [Birmingham & Kurlents (89)].

Assessment of adrenocortical activity.—The identification of the principal corticoid in each species has led to the development of methods of measuring these compounds for use in the assessment of adrenocortical activity. In the rat, the adrenal venous blood content of corticosterone was investigated as an index of adrenocortical function, but the stress of cannulation was so

severe that exogenous corticotropin had little or no effect [Holzbauer & Vogt (160)]. However, the residual steroids in the rat adrenal, although low in amount, were qualitatively similar to those in the blood [Holzbauer (161)]. This observation led to excellent correlation between the corticosterone content of the rat adrenal gland and adrenal venous blood. The application of the adrenal content of corticosterone, instead of adrenal ascorbic acid depletion, as a direct index of adrenocortical function will help to decide the validity of the conclusions based on the adrenal ascorbic acid.

Another gauge of adrenocortical function is the formation of steroids by the isolated adrenal tissue. The production of steroids by rat adrenal tissue *in vitro* depends upon prior treatment of the rats. Exposure to severe cold caused an increase in steroid production during the first half hour of incubation only [Schönbaum *et al.* (162)]. In this case, the *in vitro* stimulation of corticoid production and the *in vivo* adrenal ascorbic acid content failed to fluctuate in concert. In man there is good correlation between the production of cortisol by the adrenal *in vivo*, as estimated from adrenal venous blood, and subsequent *in vitro* production of cortisol by slices of the same gland [Cooper *et al.* (163)].

CATABOLISM OF CORTICOSTEROIDS

Absorption.—Cortisol is, of course, most readily utilized after intravenous administration. The speed of absorption by other routes decreased, in rats, in the following order: sublingual, intramuscular, intragastric [Hyde & Williams (164)]. Esterification of the hormone decreased the rate of absorption from the gut, yielding a lower, but more prolonged maximum in the blood [Collins *et al.* (165)]. The topical administration of cortisol is effective therapeutically, but the rate of absorption through the skin or from the colon is so slow that there were no detectable increases in urinary or plasma levels [Smith (166); Schwartz *et al.* (167)]. However, injections of prednisolone did cause eosinopenia, indicating that some percutaneous absorption occurred [Tschan & Adoni (168)].

Binding by protein.—Under resting conditions, practically 100 per cent of the plasma corticosteroids appears to be bound by proteins [Daughaday (169); Antoniadis *et al.* (170)]. The steroids were usually found to be associated with albumin, but the existence of a strongly-binding globulin has been suggested by Daughaday (171). The binding capacity of plasma proteins is apparently limited, for elevation of plasma corticosteroids by any means resulted in the appearance of the free compounds (169). According to Sandberg & Slaunwhite (172), protein-bound steroids are likely to be excreted in the bile. Thus, the presence or absence of a substance in the bile and feces is an indication of its binding by plasma proteins. By this token, these authors found that 11β -hydroxyandrostenedione is probably only feebly bound.

Little is known of the chemical nature of the binding between steroid and protein. The preparation by Erlanger *et al.* (173) of artificial steroid-protein

conjugates will make possible the study of properties of such compounds and may clarify the structure of the natural conjugates.

Clearance from the blood.—The rate of removal of administered corticosteroids has been determined in man and in a variety of animals. In normal human subjects given cortisone, the time for 50 per cent removal of Porter-Silber chromogens was about 60 min. However, the half life of cortisone itself was only about 30 min., suggesting that a substantial conversion to cortisol and other Porter-Silber chromogens occurred [Peterson *et al.* (174)]. The clearance of cortisol was less rapid, with a half time of approximately 100 min. The dog cleared cortisol twice as rapidly as man, and the rate of clearance was not altered by the administration of fluid, by variations in the dose of cortisol, or by moderate doses of corticotropin [Kuipers, Ely & Kelley (175)]. However, a large dose of corticotropin apparently decreased the clearance rate, perhaps by promoting the secretion of less-rapidly removed corticosteroids. Ethynyl estradiol also delayed clearance of cortisol, but there was no explanation for this effect [Wallace, Silverberg & Carter (176)]. In liver disease, the rate of clearance of cortisol was decreased markedly [Peterson *et al.* (174); Englert *et al.* (177)], supporting the long-held view that the liver is a major site of corticosteroid catabolism [Hechter and coworkers (178); (179)].

Ring A: reduction and conjugation.—A large fraction of the cleared cortisol is quickly reduced to tetrahydrocortisol and tetrahydrocortisone, which are, in turn, conjugated with glucuronic acid in the liver. The rate limiting step is probably the reduction, so that the reduced compounds are conjugated as quickly as they are formed [Brown *et al.* (180)]. Thus, the free steroids in pooled human plasma included cortisol and corticosterone, while tetrahydrocortisol and tetrahydrocortisone were found to be conjugated as glucuronides [Tyler (181); Tamm, Beckmann & Voigt (182)]. In cirrhotic liver disease the rate of reduction of cortisol to the tetrahydro compounds was decreased, but conjugation was unimpaired (177). The liver enzyme that reduces ring A required TPNH and was apparently concentrated in the microsomes [Brown, Anason & Jacobs (183)]. The major reduction products are pregnane derivatives, but smaller amounts of allopregnanes have been found [Bush & Willoughby (184); Caspi & Pechet (185, 186)].

In another form of liver disease characterized by impaired conjugation of bilirubin with glucuronic acid, the conjugation of cortisol metabolites was also reduced [Peterson & Schmid (187)]. Not only the natural corticosteroids, but also the synthetic materials, such as the Δ^1 , methylated, and halogenated analogues, are catabolized by the liver to the tetrahydro forms and then conjugated (172, 185, 186). Conjugation of cortisone and prednisone can be competitively inhibited by N-acetyl-*p*-aminophenol, resulting in a decreased rate of clearance from the blood [Corte & Johnson (188)]. According to some investigators, Δ^1 -cortisol (prednisolone) is less rapidly removed from the circulation than cortisol, and a larger proportion is excreted unchanged [Kuipers *et al.* (189); Sandberg & Slaunwhite (172)]. The greater potency of the synthetic analogues has been attributed to their

resistance to attack by liver enzymes. But there is disagreement about the rate of metabolism of the analogues of cortisol by liver tissue *in vitro* compared to cortisol itself [Brown & Anason (190); Glenn *et al.* (191)]. Another major natural corticosteroid, corticosterone, also undergoes reduction of ring A to yield tetrahydrocorticosterone [Dyrenfurth *et al.* (192)].

Changes at C-11.—Administered cortisone (11-ketone) was partially converted to cortisol(11-hydroxyl) [Baulieu & Jayle (193); Schrievers *et al.* (194)], and it has been suggested that cortisone must be converted to cortisol to be biologically active [Peterson *et al.* (174); Bush & Mahesh (195)]. On the other hand, a large proportion of the urinary metabolites of cortisol consists of tetrahydrocortisone [Cope & Black (196); Baulieu & Jayle (193)], which is more rapidly conjugated than tetrahydrocortisol (177). This suggests that some conversion of tetrahydrocortisol to tetrahydrocortisone takes place. The Δ^1 analogues of cortisol and 21-deoxycortisol also yielded 11-ketonic excretion products (185, 186, 194, 197). Rat slices can carry out the interconversion of 11-ketonic and hydroxyl compounds (194).

Reduction at C-20.—A relative excess of Δ^4 -3-ketonic steroids over Porter-Silber chromogens (dihydroxyacetone side chain) has been interpreted as indirect evidence for the presence of C-20 reduced compounds in urine [Weichselbaum, Elman & Margraf (198)]. Such compounds have actually been isolated from urine (e.g., 196, 201). The reduction takes place in liver and kidney [Glenn and co-workers (191); Brown & Anason (190); Recknagel (199); de Courcy (200)]. The enzyme, C-20-keto reductase, is concentrated in the microsomes and requires TPNH for activity (199, 200). The enzyme is relatively specific for the dihydroxyacetone side chain and only slowly attacks the 17-deoxysteroids (199).

Reduction at C-17.—Fukushima & Gallagher (197) have shown that 17-deoxy compounds arise as artifacts during acid hydrolysis and are never found after enzymatic hydrolysis.

Cleavage at C-17.—It has long been known that the side chain of corticosteroids may be lost, giving rise to 17-ketosteroids. For example, the administration of cortisone acetate in an adrenalectomized subject led to the isolation of $3\alpha,11\beta$ -dihydroxyetiocholan-17-one and 3α -hydroxyetiocholan-11,17-dione [Lombardo & Hudson (201)]. Cortisone and cortisol gave rise to both 11β -hydroxy and 11-keto 17-ketosteroids, with compounds of the etiocholine series predominating [James, Baulieu & Jayle (202)]. The Δ^1 -analogues of cortisol and cortisone are similarly converted to 17-keto compounds, but at a much slower rate, yielding the same products as the natural corticoids [Caspi & Pechet (185, 186)] or the unsaturated derivatives, $\Delta^1,4$ -androsta-3,11,17-trione and 11β -hydroxy- $\Delta^1,4$ -androsta-3,17-dione [Vermeulen (203)].

17-Ketosteroids.—The systematic study of 17-ketosteroid excretion continues, relating the urinary 17-ketosteroid level to sex, age, etc. and to the time of collection (204, 205, 206). Dehydroepiandrosterone and androsterone have been identified as the principal 17-ketosteroids in human peripheral blood, and the blood levels have been correlated with age, sex, and

time of sampling (207). 3 α -Hydroxyetiocholan-11,17-dione, 3 α -hydroxyetiocholan-17-one and 11 β -hydroxy- Δ^4 -androstene-3,17-dione have also been found in human plasma (208). Mice, bearing corticotropin-producing pituitary tumors, excreted mainly 11- β -hydroxy- Δ^4 -androstene-3,17-dione and its saturated analogue (157). In guinea pig urine, a large variety of 17-ketosteroids was detected (209).

A considerable fraction of the urinary 17-ketosteroids arises in the gonads and appears to be stimulated by the administration of chorionic gonadotropin (210). Androgens apparently suppressed the excretion of 17-ketosteroids by an action on both the adrenal cortex and the gonads (211).

Bradlow & Gallagher (212) have studied the metabolism of ^{14}C -labelled 11 β -hydroxy- Δ^4 -androstene-3,17-dione in man. It was rapidly converted to 3 α -11 β -dihydroxyandrostane-17-one as the major product with lesser amounts of the 11-keto compound and the corresponding etiocholan epimers. The equilibrium between the 11-hydroxyl and carbonyl group was studied more closely by Bush & Mahesh (195), who observed that only compounds of the androstane series entered such an equilibrium, while etiocholan derivatives were excreted unchanged. As in the case of the estrogens, 17-ketosteroids can be hydroxylated at C-16, dehydroepiandrosterone forming the 16 α -hydroxy derivative (213).

There are no unconjugated 17-ketosteroids in urine; both sulfates and glucuronides are excreted. Development of methods for the separation of conjugates has made their characterization possible. Dehydroepiandrosterone sulfate accounts for most of the sulfate fraction, while the glucuronide fraction contains approximately equal amounts of androsterone and etiocholanolone as well as the 11-oxygenated 17-ketosteroids. The 3 β -hydroxy compounds are excreted as sulfates [Crepy *et al.* (214)]. The mechanism of conjugation with sulfate is now better understood, and the liver enzymes involved are being characterized [DeMeio *et al.* (215); Robbins & Lipmann (215); Roy (217)].

Excretion in bile.—Only small amounts of corticosteroids (174, 218) and the 17-ketosteroids (219) were found in the bile.

Excretion in urine.—Most metabolites of the adrenocortical steroids are excreted into the urine. The rate of excretion, in guinea pigs, is correlated with urine volume in a diurnal variation. But, under conditions of adrenocortical stimulation such as after corticotropin, the excretion is independent of volume [Lazo-Wasem & Hier (220)]. Steroid glucuronides are excreted mainly by glomerular filtration, resulting in very low plasma levels, while sulfates are either partially reabsorbed or are excreted by the renal tubules, with a consequent slower rate of clearance. Hence, the blood 17-ketosteroids are predominantly sulfates [Kellie & Smith (221)]. The clearance of glucuronides by hypertensive patients with normal renal function was claimed to be only one-half that of normotensives [Kornel (222)]. In cases of chronic renal disease the clearance of adrenocortical steroids decreased in proportion to the severity of the disease [Englert *et al.* (223)].

Pregnanols.—Like the 17-ketosteroids, the urinary pregnanols arise in both the gonads and the adrenal cortex. The evidence for the adrenal origin is the increased excretion of pregnandiol and pregnantriol after the administration of corticotropin to ovariectomized women [Nabarro & Moxham (224); Klopper, Strong & Cook (225)]. These compounds are believed to arise as metabolites of progesterone and 17-hydroxyprogesterone, which are intermediates in the biosynthesis of adrenocortical hormones (Fig. 1). In certain adrenal hyperplasias, characterized by a disturbance in the conversion of the intermediates to cortisol and corticosterone, the intermediates accumulate, and their metabolites are excreted in elevated quantities (e.g., 150, 227).

ADRENAL DISEASES

Categories.—Adrenal diseases can be divided into several functional categories: (a) underproduction of all adrenal hormones—Addison's disease; (b) overproduction of all adrenal hormones—Cushing's syndrome; (c) overproduction of single hormones—aldosteronism; (d) enzymatic disturbance, with overproduction of intermediates in hormone synthesis or of usually minor products, associated with a lack of true hormones—congenital adrenal hyperplasia, adrenogenital syndrome, virilism, feminism, etc. As discussed previously, the enzymatic disturbance can occur at any point in the biosynthesis of the hormones (Fig. 1). In the adrenogenital syndrome, several main disturbances have been found: a deficient hydroxylation at C-11, with increased excretion of 11-deoxy-17-hydroxycorticosterone and its metabolites [e.g., Dyrenfurth *et al.* (192)]; impaired hydroxylation at C-21, with increased excretion of the pregnanols (150, 227); a metabolic block favoring the overproduction of 17-ketosteroids [Jaoudé, Baulieu & Jayle (226); Masouda (227)].

Hypofunction.—In classical Addison's disease, basal levels of plasma and urinary corticosteroids are low and are not increased by the administration of corticotropin [e.g., Appel (228)]. However, it is becoming apparent that some adrenocortical insufficiency can coexist with normal resting values, signs of Addison's disease only becoming manifest under conditions of stress which exhaust the limited adrenal reserves. Such cases are characterized by normal resting corticosteroid values, which fail to increase in response to corticotropin [Martin *et al.* (229); Haydar *et al.* (230); Mandelstram *et al.* (231)].

Hyperfunction.—This can be caused by hyperplasia, or by a functional tumor. As a general rule, the hyperplastic adrenal can be stimulated by corticotropin, usually excessively [Sandberg and co-workers (232); Soffer, Geller & Gabilove (233)], whereas the hyperfunctioning tumor is often independent of corticotropin [Sandberg *et al.* (232); Soffer *et al.* (233); Jenkins & Spence (234)]. Another way of determining dependency on corticotropin in hyperfunctional states is the suppression of plasma and urinary corticosteroids by the administration of natural or synthetic corticoids (232, 234, 235).

Zondek, Zondek & Leszynsky (236) repeated the warning against the use of a single unsupported determination of corticoids in the diagnosis of adrenal disease. Bloom (237) has reviewed tests of adrenocortical function.

EFFECTS OF CORTICOTROPIN

Mode of action.—The hypothesis that corticotropin influences adrenocortical function via an effect on adrenal phosphorylase received further attention. Haynes (238) found that corticotropin increased the concentration of cyclic adenine nucleotide in adrenal tissue *in vitro*. The cyclic nucleotide activates phosphorylase, which in turn increases available TPNH. Koritz & Péron (239) confirmed that additional supplies of TPNH, furnished by adding TPN⁺ plus suitable substrates, stimulated corticosteroid production by rat adrenals *in vitro*. However, TPNH activated corticosteroid formation even in tissue maximally stimulated by corticotropin, and TPNH promoted corticosteroid synthesis by frozen and thawed tissue, whereas corticotropin had no effect. Apparently, therefore, TPNH and corticotropin have different modes of action.

Steroid synthesis.—The response of rat adrenals *in vitro* to corticotropin was found to be proportional to the log dose per unit adrenal weight and independent of the concentration of corticotropin in the medium. Short exposures (5 min.) of adrenal tissue to corticotropin solutions resulted in a stimulation of corticosteroid formation by the tissue, and a corresponding loss of corticotropin activity in the solution. These observations were explained by a binding of corticotropin by the adrenal tissue [Birmingham & Kurlents (89)].

Certain transplantable functional tumors in mice and rats were also active *in vitro*, forming a variety of steroids and responding to corticotropin [Cohen, Bloch & Celozzi (240)]. Like normal adrenal tissue, the tumors responded best to corticotropin in the presence of glucose, but were more sensitive to corticotropin. Another adrenal tumor in mice was characterized by high concentration of 17-ketosteroids in the plasma, but its function was uninfluenced by corticotropin [Poore & Hollander (241)]. The dog adrenal gland, perfused *in situ*, was successfully used by Hilton *et al.* (242) to demonstrate that, in the hypophysectomized animal, as little as 1 mU of corticotropin stimulated cortisol formation fourfold.

The action of corticotropin in rats was potentiated by the administration of squalene, but apparently without influencing the synthesis of cholesterol by the adrenal gland [Kline (243)]. On the other hand, estrogens and gonadotropins diminished the response to corticotropin in guinea pigs [Clayton & Hammant (244)].

Ascorbic acid.—One of the most interesting problems in adrenal physiology is the puzzle of the role of ascorbic acid. What is its origin? Why is it present in such high concentrations? Where does it go? Only partial answers are available.

The adrenal ascorbic acid is apparently not synthesized in the gland, but probably is removed from the blood [Salomon (245); Dayton and Burns

(246)]. Salomon (247, 248) believes that intracellular ascorbic acid, in erythrocytes and in the adrenal cortex, exists in two forms, distinguished by the rapidity of exchange with plasma ascorbic acid. According to Salomon, only the slowly exchangeable form is depleted by corticotropin, accounting for the fact that the maximum depletion in the gland is only about 50 per cent. The actual participation of ascorbic acid in steroid biosynthesis or in the action of corticotropin is not understood at all, and is only sporadically studied. However, the fate of the ascorbic acid after corticotropin has been examined and correlated with steroid formation. Salomon (249) administered ^{14}C -ascorbate to hypophysectomized rats and identified all the ^{14}C in the adrenal gland as ascorbate. After corticotropin, ^{14}C entered the adrenal venous blood. The release of ^{14}C ascorbate by corticotropin could be imitated by stopping the adrenal circulation momentarily. Slusher & Roberts (250) and Briggs & Toepel (251) determined the ascorbic acid content in the adrenal venous blood of the hypophysectomized rat and found a fairly good agreement between the amount disappearing from the tissue and appearing in the blood. Both groups of workers observed that stress in hypophysectomized animals led to a detectable increase in the ascorbic acid content of aortal or cardiac blood, although stress did not change the biological half life of ascorbate [Salomon (252)].

There is general agreement that the time course of the depletion of adrenal ascorbic acid after corticotropin or stress differs from that of the secretion of corticosterone (251, 253, 254). The level of adrenal ascorbic acid did not always reflect other indices of adrenal function (162). Slusher & Roberts (250) found that while corticosterone secretion proceeded at a more or less linear rate, the loss of adrenal ascorbic acid was very rapid in the first 15 min., after which it leveled off. The authors suggested that corticotropin exerts its effects on ascorbic acid and corticosteroid synthesis by independent mechanisms. It should be pointed out, however, that the adrenal stores of preformed corticosterone are negligible, so that the rate of release from the tissue would be comparable to the rate of synthesis, while the level of adrenal ascorbic acid is high, and large amounts can be released quickly. Guillemín *et al.* (253, 254), contrary to Slusher and Roberts, found that significant increases in the rate of corticosterone secretion can be observed after small doses of corticotropin at times when there was little or no change in adrenal ascorbic acid.

Adrenal weight.—The relationship between adrenal and body weight was superbly analyzed by Heroux & Gridgeman (255), who recommended that changes in the adrenal gland should be expressed in terms of absolute adrenal weight, rather than as a function of body weight. In male chickens, corticotropin exerted the same effects as in mammals except that it had little influence on adrenal weight [Brown, Brown & Meyer (256)]. Corticotropin failed to influence the incorporation of ^{14}C -glycine into rat adrenal proteins [Koritz, Péron & Dorfman (257)].

The role of growth hormone was re-examined by Lostroh (258, 259), who observed that, while growth hormone alone had scarcely any effect on

adrenal weight in hypophysectomized rats, the hypertrophy after corticotropin was enhanced by growth hormone. This observation suggests that the "adrenal weight factor", thought to represent a distinct entity, may be a preparation of corticotropin contaminated with growth hormone.

Metabolism of adrenal tissue.—The incorporation of ^{32}P into the inorganic phosphate of the adrenal gland was stimulated by corticotropin in the hypophysectomized animals, and Nicholls & Graham (260) have proposed the use of this phenomenon as an index of adrenocortical activation. Corticotropin also increased the aliesterase activity in the zona fasciculata of the mouse adrenal gland, but did not influence the activity in the zona glomerulosa [Allen (261)]. Adrenal succinoxidase activity was increased by corticotropin and still further increased by testosterone [Kalant (262, 263)]. The uniform distribution of magnesium in the adrenal gland was undisturbed by corticotropin [Glick (264)].

Extra-adrenal effects.—Engel (265) has continued his work on the ketogenic activity of corticotropin which is manifest in the adrenalectomized animal. This extra-adrenal action was found with peptide preparations of corticotropin and not with the older "protein" preparations (266). The explanation for the difference was that the protein corticotropin is selectively fixed by the adrenal, but that the peptide preparations escape binding by the adrenal and are able to exert an effect on other tissues. Engel (267) proposed that the ability of corticotropin to mobilize the lipides of extra-adrenal tissues is analogous to its action on corticosteroid biosynthesis in the adrenal gland. Higginbotham & Dougherty (268) found that corticotropin, and other basic peptides, have an affinity for heparin strong enough to displace other compounds from combination with heparin. These authors suggested that this phenomenon may be the key to the problem of the extra-adrenal action of corticotropin.

EFFECTS OF CORTICOSTEROIDS

Carbohydrate metabolism.—The undoubted influence of corticosteroids on carbohydrate metabolism still remains unexplained. One of the most consistent effects of corticosteroids is the deposition of liver glycogen. Langley & Gunthorpe (269) claimed that the hypophysis and insulin are both required, in addition to corticosteroids, for deposition of liver glycogen. The effect on glycogen in other tissues is variable. Cortisol and cortisone increased glycogen in skeletal muscle [Leonard (270)], but not in the uterus [Kostyo (271)] or in the kidney [Froesch, Ashmore & Renold (272)], while cardiac glycogen was apparently decreased under certain conditions [Gambassi & Maggi (273)]. Cortisone had no effect on the phosphorylase of skeletal muscle [Leonard (270)].

Cortisone causes impaired glucose tolerance. However, fructose and galactose tolerance were found to be unchanged [Papper, Saxon & Alpert (274)]. In the dog, the impaired glucose tolerance was followed quickly by improved glucose tolerance, probably a result of stimulation of insulin secretion [Demanet *et al.* (275)]. Deposition of liver glycogen and decreased

glucose tolerance can result from either decreased utilization or increased production of glucose or both. Altszuler *et al.* (276, 277) showed that cortisol restored to normal the decreased production of glucose by the liver in hypophysectomized dogs. Ashmore *et al.* (278) found that adrenal activity did not influence the rate of utilization of ^{14}C -glucose by liver slices, and they suggested that corticosteroids increased the production of glucose by increased gluconeogenesis. Rosen *et al.* (279) observed that glucocorticoids stimulated markedly the activity of glutamic-pyruvic transaminase in liver. They proposed this increase as the enzymatic basis for the gluconeogenic action.

The glycosuria following administration of glucocorticoids results from the excessive load of glucose caused by a combination of hyperglycemia and increased filtration rate [Froesh *et al.* (280)]. There seems to be no specific effect of cortisone on carbohydrate metabolism by the kidney [Froesch, Ashmore & Renold (272)].

The effects of corticosteroids on carbohydrate metabolism resemble, in several ways, the picture seen in diabetes mellitus, and, in some species, overdosage with corticosteroids has caused a more or less permanent diabetic state. The cat was seemingly resistant to the development of "steroid diabetes", but Buse, Gundersen & Lukens (281) succeeded in making cats diabetic with the potent synthetic steroid, 9α -fluorocortisol. In the diabetic animals there was hydropic degeneration of the islets in the pancreas.

In the diabetic, the anti-insulin action of the corticosteroids is unopposed, and serum from alloxan-diabetic rats was found to inhibit the uptake of glucose by diaphragm *in vitro*. This observation was again confirmed by Whitney & Young (282). Hypophysectomy of the diabetic animal resulted in loss of the inhibitory properties of the serum, which could be restored by treatment of the animal with growth hormone and cortisone, but not by adding these hormones *in vitro*. Treatment of normal animals with growth hormone and cortisone caused a slight decrease in the uptake of glucose by the diaphragm. Very similar results were obtained by Vallance-Owen & Lukens (283) in the cat. The insulin-inhibitor in diabetic serum appeared to be concentrated in the α -globulins. However, in Cushing's syndrome, diminished glucose uptake and insulin resistance could not be demonstrated [Fabrykant, Jackson & Ashe (284)].

Carbohydrate materials, other than glucose, are also affected by corticosteroids. The formation of mucin, as measured by ^{35}S uptake by the stomach, was reduced by corticosteroids [Denko (285)]. Some of the treated animals developed ulcers [but see Lehr *et al.* (286)]. Cortisone also diminished the uptake of ^{35}S in growing bones of cockerels [Kowalewski (287)]. Another sulfur-containing carbohydrate, heparin, was reduced in quantity in the skin by treatment with cortisone [Monkhouse, MacKneson & Bambers (288)].

Nitrogen metabolism.—Corticosteroids produce the well-established loss of nitrogen, some of which appears as purine catabolites in the urine. X-irradiation also caused the appearance of greater-than-normal amounts of purine catabolites, and the effect persisted after adrenalectomy [Jackson & Entenman (289)]. Corticosteroids and chlorpromazine produced additive

loss of nitrogen [Rupp & Paschkis (290)]. These observations add to the evidence of the extra-adrenal influence of stressors on nitrogen metabolism. Some of the nitrogen lost after stress or the administration of corticoids arises from the protein liberated by destruction of lymphatic tissues and plasma cells [Dolowitz *et al.* (291)]. The liberated protein is first detectable as an increase in serum protein levels, especially in the γ -globulin fraction containing antibodies [Dolowitz *et al.* (291); Schultz *et al.* (292); Scheiffarth *et al.* (293)]. Even before the breakdown of lymphatic cells begins, cortisone was found to decrease CO_2 production and the incorporation of ^{14}C -precursors into lymphatic tissue proteins. Shewell (294) has determined the thymolytic activity of various native and artificial corticosteroids.

Cortisol promotes the concentration of ^{14}C -labeled α -aminoisobutyric acid, a synthetic nonmetabolizable amino acid, by the liver [Noall *et al.* (295)]. Cortisone decreased blood levels of proline in rats, without influencing its excretion [Kivirikko & Liesmaa (296)], suggesting the entry of proline into tissues. Liver arginase, an enzyme of protein catabolism, was increased by cortisone [Bach, Carter & Killip (297)]. These studies support the concept that corticosteroids promote the utilization of amino acids and proteins for gluconeogenesis. The survival of adrenalectomized rats was dependent not only on maintenance with cortisone, but also on the level of dietary protein [Wolf (298)].

Eosinophils.—The well known effect of corticosteroids on eosinophilic leucocytes is still being studied. Hudson & Doig (299) inferred from the linear rate of eosinopenia that corticosteroids caused a stoppage in the supply of new cells with a constant elimination of those already in the blood. They estimated the life span of the eosinophil as lying in the range of 165 to 377 min.

ALDOSTERONE

Aldosterone has emerged as the chief, if not the only, natural mineralo-corticoid. It is formed exclusively in the zona glomerulosa via a biosynthetic pathway that is becoming clear.

Primary control of aldosterone secretion.—It is now well established that the secretion of aldosterone by the zona glomerulosa is substantially free of pituitary control. However, growth hormone caused an increase in aldosterone excretion, without influencing the excretion of other adrenal steroids [Beck *et al.* (51)]. Farrell *et al.* (300) tested purified corticotropins for ability to provoke aldosterone synthesis in the decerebrate dog and found that δ_1 -corticotropin was several times as active as β -corticotropin. Both preparations were equally effective in stimulating the production of cortisol. They suggested that the variable results of others with less pure preparations of corticotropin might be accounted for by the differing content of δ_1 -corticotropin. In spite of the activity of these adeno-hypophysial hormones, the search for the primary mediator of aldosterone secretion is concentrated on the diencephalon. Farrell's group [Newman *et al.* (301)] has explored the reticular formation in the midbrain and brain stem with a coagulating

electrode, seeking areas whose destruction would alter the secretion of aldosterone and cortisol in the cat. Extensive destruction in the diencephalon reduced the output of both hormones. A lesion in the pons actually increased aldosterone secretion, leaving cortisol unchanged. Thus there is some evidence for a dissociation between the stimuli to mineralo- and glucocorticoids and for an aldosterone restraining center at the level of the midbrain.

Daily & Ganong (302) have tried to correlate the location of lesions in the hypothalamus of the dog with changes in electrolytes. Lesions causing diabetes insipidus, gonadal atrophy or failure of the adrenocortical response to stress or both, were without detectable effect on sodium and potassium metabolism, even in response to a salt load or to salt restriction. While the lack of effect on electrolytes is suggestive, it does not rule out the possibility that some of the lesioned animals actually had altered aldosterone secretion.

There is little additional evidence for the existence of an aldosterone-stimulating factor in the brain, although Giroud *et al.* (303) have observed that certain preparations of posterior pituitary hormones, added *in vitro* to adrenal tissue, stimulated the formation of aldosterone. Intact and adrenalectomized rats deprived of salt excreted a thermolabile, nondialyzable, butanol extractable material, which, administered to hydrated rats, stimulated the excretion of aldosterone in the feces and caused decreased urinary excretion of sodium. Corticotropin did not have similar properties [Orti *et al.* (304)]. Experiments to determine the origin of this material are eagerly awaited. A direct influence of circulating electrolytes on aldosterone production was postulated in the past, but this concept has failed to gain experimental support.

Secondary control of aldosterone.—If a diencephalic factor is assumed to be the primary effector of aldosterone secretion, two secondary factors, perhaps related, have been proposed to influence the release of the diencephalic factor: (a) serum electrolyte balance; and (b) changes in the partition and amount of body water in the intra- and extracellular compartments.

(a) Sodium deficiency in rats increased the formation of aldosterone by the adrenal glands *in vitro* and decreased the production of other corticosteroids [Eisenstein & Hartroft (305)]. At the same time there were signs of hyperactivity in the zona glomerulosa, with atrophy of the fasciculata, which the authors attributed to a crushing of the fasciculata by the expanding glomerulosa within the confines imposed by the adrenal capsule [Hartroft & Eisenstein (306)]. In dogs, however, a low-sodium diet caused no detectable change in the zona glomerulosa [Race *et al.* (307)]. In human subjects, dietary restriction of potassium decreased urinary excretion of aldosterone, while restriction of sodium increased it; the simultaneous restriction of both ions resulted in a delayed increase in aldosterone excretion, which was not as pronounced as that with sodium restriction alone [Johnson, Lieberman & Mulrow (308)]. While it is possible that changes in electrolytes influence aldosterone formation directly, restriction of electrolytes is inseparable from shifts of water which may actually be responsible for the release of the postulated aldosterone-stimulating factor (309).

(b) The mobilization of the postulated aldosterone-secreting factor may be caused by a reduction in the volume of extracellular fluid, which would be detected by a volume receptor, probably in the head (309). Acute hypovolemia after bleeding caused a substantial increase in the excretion of aldosterone [Fine, Meiselas & Auerbach (310)]. Shifts in the distribution of water can be caused by standing up and lying down. Standing resulted in a flow out of the vascular system, and in sodium retention; lying down caused the opposite effects [Thomas (311)]. Such postural effects on salt and water metabolism were not found in patients with Addison's disease [Taymor & Friedberg (312)].

Constriction of the thoracic inferior vena cava in dogs resulted in ascites and increased aldosterone output [Davis *et al.* (313)]. Hypophysectomy caused a fall in aldosterone secretion in normal dogs and in dogs with ascites, but did not prevent the increase that followed the development of ascites. The above studies, using urinary aldosterone, were confirmed in experiments in which the aldosterone in adrenal venous blood was measured [Davis *et al.* (314)]. Constriction of the inferior vena cava just above the entrance of the adrenolumbar veins did not result in ascites, nor in increased aldosterone secretion [Ball & Davis (315)], demonstrating that the constriction per se was not responsible for the effect on the adrenal, but rather the shifts in water associated with hepatic congestion and ascites. When ascites was prevented after hepatic congestion by the application of a body cast, there was no stimulation of aldosterone excretion [Davis & Ball (316)].

In man the counterpart of these experiments is seen in liver disease. Increased excretion of aldosterone followed the loss of vascular fluid that accompanies the formation of ascites [Wolff *et al.* (317, 318, 319)]. These authors, in excellent papers, attempt to correlate adrenal and neurohypophyseal involvement in the fluid shifts that occur in normal subjects and in patients with cirrhosis. The same group has studied aldosterone excretion after fluid changes occurring in heart disease (318). Many other studies were carried out on aldosterone output in cardiovascular disease [e.g., Genest *et al.* (320) on human arterial hypertension, and Driscoll *et al.* (321) on experimental congestive heart failure in dogs]. The excretion of aldosterone in hypertensive patients was uniformly greater than normal. In experimental congestive heart failure induced by pulmonary artery constriction, there was no increase in the secretion of aldosterone in adrenal vein blood. This observation conforms to the results of Wolff *et al.* (318) in which patients with "left-sided" heart failure had normal aldosterone values, in contrast to the elevated values in patients with "right-sided" failure.

The influence of water shifts on aldosterone output may explain the effects of vasopressin on aldosterone secretion. The administration of vasopressin causes antidiuresis, and a retention of water in the circulatory system, leading to a diminution of aldosterone output. The subsequent release of the retained fluid suddenly shifts water out of the vascular system, causing an increased secretion of aldosterone which prevents an inordinate loss of sodium along with the water. An example of this phenomenon was

seen after hypophysectomy performed in a patient with cancer of the breast. Aldosterone excretion increased after the operation during the period of diabetes insipidus, and returned to normal after treatment with vasopressin [Little *et al.* (322)].

Girerd & Green (323) reported the presence of a sodium-retaining material in the urine of adrenalectomized hypertensive patients maintained with cortisone. They speculated that the sodium-retaining factor could originate in adrenal remnants, in nonadrenal tissue, or from a conversion of the administered cortisone into a metabolite with mineralocorticoid activity. Share & Stadler (324) reported sodium retention and potassium loss in adrenalectomized rats after trauma and thus supported the concept of an extra-adrenal sodium-retaining factor. In adrenalectomized dogs, hypokalemia after stress still occurred [Ferguson, Smith & Barry (323a)]. The involvement of the neurohypophysis and the adrenal cortex in electrolyte and water metabolism was recently reviewed by Dingman (325).

Hyper- and hypoaldosteronism.—Hypersecretion of aldosterone causes the now well-characterized disease, primary aldosteronism. Usually this disease is associated with polyuria and absence of edema, because compensating mechanisms attempt to eliminate the sodium retained by the action of aldosterone through increasing the flow of urine. Richter (326) suggested that the hypokalemia in aldosteronism decreases the responsiveness of the kidney to antidiuretic hormone, resulting in polyuria, which prevents edema. However, a case of primary aldosteronism associated with edema and hypertension has been reported by Goldsmith *et al.* (327). All signs of the disease, including the edema, disappeared on the removal of an adrenocortical adenoma.

Just as there are diseases of hyper- and hyposecretion of the glucocorticoids, hypo- as well as hyperaldosteronism may theoretically exist. Skanse & Hökfelt (328) described a case of "pure" hypoaldosteronism, unassociated with signs of hyposecretion of other adrenocortical steroids. The authors suggested that the disease's apparent rarity was attributable to lack of recognition and that a review of patients with unexplained hypotension or hyponatremia, or both, might reveal more examples of the syndrome.

Effects of adrenalectomy on salt and water.—Adrenalectomy is followed by a decrease in tissue sodium and an increase in tissue potassium [e.g., Efron (329)]. Such changes may result from a specific effect on the kidney, with changes in other tissues being secondary to the excretion of sodium and retention of potassium. However, the tissue changes may also result from an effect on the permeability of all cells to the ions. The extrarenal effect was studied in nephrectomized rats by Friedman, Nakashima & Friedman (330). Adrenalectomy in such rats resulted in a fall in blood pressure, decreased inulin (extracellular) space and a decreased extracellular sodium concentration to attain a new equilibrium at lower levels. The authors attribute the shift to compensatory neurohypophysial hyperfunction, which moves water and sodium out of the extracellular space. The movement of ions and water after adrenalectomy was also reflected in the milk of the

lactating goat [Cowie & Tindal (331)]. Milk secretion (i.e., water content) fell, along with the potassium concentration, while the sodium content rose slightly.

Effects of mineralocorticoids.—On the assumption that their mechanism of action is similar [but see (332)], the effects of natural and artificial mineralocorticoids will be considered together. The mineralocorticoids have the opposite effect to adrenalectomy on salt and water metabolism. That is, they promote retention of sodium, and excretion of potassium and water. The administration of deoxycorticosterone increased the absorption of ^{24}Na by all tissues studied, except the kidney [Valuera (333)]. Deoxycorticosterone glucoside, added to a suspension of red blood cells, caused a fall in cell potassium and an increased cell sodium [Sherwood Jones (334)]. The same effect was observed in leucocytes by Wilson (335). The source of the increased tissue concentrations of sodium was studied by Sweet, Levitt & Hodes (336). The diet, bone, and tendon together did not account for the retained sodium, and the authors suggested that the gastrointestinal tract was a likely source of the salt.

The site of action of aldosterone was studied by Nicholson (337). Damage to the distal tubules of the kidney with mercuric chloride failed to affect the action of either deoxycorticosterone or aldosterone on the excretion of sodium, while damage to the proximal convoluted tubules with sodium tartrate prevented the retention of sodium by these hormones. This elegant experiment helps to identify the kidney as the main target tissue of the mineralocorticoids.

The action of both deoxycorticosterone and aldosterone on sodium retention was prevented by the administration of two synthetic steroids, 3-(3-oxo-17 β -hydroxy- Δ^4 -androst-17 α -yl) propionic acid γ -lactone and its 19-nor analogue, both in adrenalectomized rats [Kagawa, Cella & Van-Arman (338)], and in patients with congestive heart failure or in Addison's disease, in whom sodium excretion resulted [Liddle (339)].

In general, the synthetic steroids with glucocorticoid activity, as well as the natural glucocorticoids, caused diuresis [Lichtlen (340), Raisz *et al.* (341); Kovács *et al.* (342)]. Glucocorticoids did not prevent the action of the antidiuretic hormone (342), and probably affected water metabolism by a direct action on the kidney (341). For reviews on aldosterone, see Neher (343); Soffer *et al.* (344); and *An International Symposium on Aldosterone* (345).

HYPERTENSION

In spite of some early conflicting reports, it is now agreed that chronic treatment with aldosterone of rats sensitized by unilateral nephrectomy and extra NaCl results in hypertension [Kumar *et al.* (346); Gross, Loustalot & Meier (347)]. The hypertensive effect of aldosterone was more pronounced in male animals [Cornall, Grundy & Koladich (348)]. In hypertension induced by deoxycorticosterone, increased amounts of both sodium and potassium were found in aortal tissue; the increases were roughly proportional

to the severity of the hypertension [Tobian & Redleaf (349)]. Restriction of sodium prevented the development of hypertension after treatment with deoxycorticosterone (349, 350, 351), but not after treatment with cortisone (350, 351). Deoxycorticosterone hypertension, but not cortisone hypertension, was accompanied by a loss of carcass potassium and a gain of sodium [Knowlton & Loeb (350)]. The synthetic steroids with predominantly mineralocorticoid properties produced hypertension with the characteristics of deoxycorticosterone hypertension, while those with glucocorticoid properties produced a hypertension like that of cortisone (351). Little is known of the way in which the corticosteroids produce hypertension. Dunihue & Robertson (352) suggested that the steroids regulate the granulation (i.e., secretory activity) of the juxtaglomerular apparatus in the kidney. Treatment with deoxycorticosterone decreased granulation, and adrenalectomy increased it.

Hypertension is produced without steroid treatment in the Skelton rat, that is, uninephrectomized, and uniadrenalectomized, with enucleation of the remaining adrenal gland. According to Neff & Correll (353), only female rats developed this type of hypertension. Testosterone prevented the regeneration of the enucleated adrenal and the development of hypertension. The prevention of regeneration with the adrenolytic drug, amphenone, also prevented hypertension in the Skelton rat, but not in the deoxycorticosterone-treated rat [Chappel *et al.* (354, 355)]. The regenerating rat adrenal was found to form the same kind of corticosteroids as the normal gland, suggesting that the development of the hypertension involves a sensitization of the vasculature, during the period of hypofunction, to the normal steroids rather than a production of abnormal hypertensive substances [Masson, Koritz & Péron (356); Skelton (357)]. Skelton rats, as well as animals treated with steroids, developed lesions in the kidney and in the cardiovascular system. These lesions were more severe and more numerous in rats given renin [Masson, Corcoran & Page (358)].

The relationship between the mineralocorticoids and hypertension has led to speculation on the role of aldosterone in the development of human hypertensive disease. Genest *et al.* (320) found consistently elevated aldosterone excretion in hypertensive patients, and Wolff *et al.* (318) have discussed the reciprocal effects of heart disease and aldosterone production. Adrenalectomy has been employed in the treatment of severe arterial hypertension, but is only recommended in carefully selected cases [Wolferth *et al.* (359)].

PREGNANCY

During pregnancy there are elevated blood levels of corticosteroids [e.g., Birke *et al.* (360); Kawahara (361)]. An increase in circulating corticosteroids may result: (a) from production by extra-adrenal tissues, such as the placenta; (b) from a higher rate of formation in the adrenal cortex; (c) or from a decreased rate of clearance from the blood. The pregnant adrenalectomized or Addisonian woman offers an opportunity for deciding among

these alternatives. Baulieu *et al.* (362) could not detect any cortisol in the plasma of an Addisonian, ruling out a major formation of cortisol itself by the placenta. However, urinary excretion of pregnanetriol was increased in pregnancy, even after adrenalectomy, pointing to the placenta as the site of production of progesterone or 17 α -hydroxyprogesterone [Herrmann & Silverman (363)]. These or similar substances may serve to increase the supply of precursors of corticosteroids, for conversion to corticosteroids by the adrenal cortex, or, more slowly, by other tissues [Herrmann & Silverman (363); Appleby & Norymberski (364)]. The extra-adrenal formation of corticosteroids from placental precursors, if actual, would account for the improvement of the Addisonian woman in pregnancy [Schüller (365)], although corticosteroids from the fetal adrenal may also contribute [Christianson & Chester Jones (366)].

There is no direct evidence for an increased production of corticosteroids by the adrenal cortex during pregnancy. However, there is some evidence that altered metabolism may contribute to both the increased plasma levels and sensitivity to corticotropin. Firstly, the increased excretion of corticosteroids in pregnancy was not as great as might be expected from the elevated plasma levels [Appleby & Norymberski (364); Martin & Mills (367)]. Secondly, the half life of cortisol was decidedly increased in pregnancy [Migeon, Bertrand & Wall (368); Wallace, Silverberg & Carter (176)]. An inhibition by estrogens of the catabolism of cortisol in the liver was been suggested as an explanation [Martin & Mills (367); Wallace, Silverberg & Carter (176)].

The high plasma corticosteroid levels were still further increased by the stress of labor [Kawahara (361)]; Little, Vance & Rossi (369); McKay, Assali & Henley (370)]. The excretion of aldosterone was also increased during normal pregnancy, and was essentially absent in the Addisonian [Nowaczynski, Koiv & Genest (371); Laidlaw, Cohen & Gornall (372); Baulieu *et al.* (362)].

THYROID-ADRENOCORTICAL RELATIONSHIP

Effects of thyroid on adrenal.—There is agreement that the rate of metabolism of corticosteroids is dependent on the level of thyroid function [Peterson *et al.* (174); Peterson (373); Brown, Anason & Jacobs (183); Jakobson (374); Williams, Crispell & Parson (375); Brown, Englert & Wallach (376); Aterman (377)]. In thyrotoxicosis, or after the administration of thyroid hormones, the rate of clearance of corticosteroids from the blood and urinary excretion of metabolites increases, whereas in myxedema or after thyroidectomy, there is a tendency towards decreased rate of metabolism of corticosteroids. The site of action of the thyroid hormones is probably the liver (183, 373).

Thyroid hormones enhanced the adrenal weight-stimulating effect of growth hormone [Lostroh & Li (378); Bois & Selye (379)], and hypothyroidism caused by thiouracil decreased adrenal weight [Roy, Karkun & Sur (380)]. However, thyroid hormones did not influence the reactivity of the

adrenal gland to corticotropin [Kowalewski (381); Mikulay & Nemeth (382); but see Brooks *et al.* (383)].

Effects of corticosteroids on thyroid.—Contrary to reports by others, Beck (384) claimed that the administration of cortisone, but not corticotropin, increased the rate of ^{131}I -discharge from the rat thyroid gland. Cortisone apparently did not block the response of the thyroid to thyrotropin, nor did it influence the metabolism of ^{131}I -thyroxine. The significance of this controversial claim must await its confirmation. Bastenie & Ermans (385, 386) found that cortisone prevented some of the metabolic effects of thyroxine, but not of triiodothyronine, and they suggested that cortisone blocks the deiodination of thyroxine to triiodothyronine. According to Knigge, Goodman & Solomon (387), the renal elimination of ^{131}I is independent of the adrenal gland.

Some authors failed to find an antagonism between the hormones of the thyroid and the adrenal cortex [Fremont-Smith, Iber & Plough (388); Doisy & Lardy (389)]. However, Denhoffer *et al.* (390) claimed that in hypophysectomized rats, cortisone prevented the increase in oxygen consumption by triiodoacetic acid. It appears that the influence of the hypophysis on the interaction between the adrenal cortex and the thyroid merits further study. Hetzel, Williams & Lander (391) concluded that neither triiodothyronine nor cortisol alone reproduced, in man, the metabolic changes after stress and suggested that both hormones together yielded a more faithful picture of the effects of stress.

LITERATURE CITED

1. Montandon, A., *Arch. Pathol. Anat. u. Physiol., Virchow's*, **330**, 629–50 (1957); *Chem. Abstr.*, **52**, 6536 (1958)
2. Rochefort, G. J., and Saffran, M., *Can. J. Biochem. and Physiol.*, **35**, 471–80 (1957)
3. Farquhar, M. G., *Anat. Record*, **127**, 291 (1957) (abstract)
4. Wolstenholme, G. E. W., and Millar, E. C. P., Editors, *Ciba Foundation Colloq. on Endocrinol.*, **11**, 416 pp. (1957)
5. Loraine, J. A., *Clinical Application of Hormone Assay* (E. & S. Livingstone, Ltd., Edinburgh and London, 368 pp., 1958)
6. Persky, H., and Heath, H. A., *J. Clin. Endocrinol. and Metabolism*, **17**, 632–44 (1957)
7. Van der Vies, J., *Acta Physiol. et Pharmacol. Neerl.*, **5**, 361–84. (1957)
8. Loraine, J. A., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 19–37 (1957)
9. Clayton, B. E., Hammant, J. E., and Armitage, P., *J. Endocrinol.*, **15**, 284–96 (1957)
10. Roberts, S., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 167–92 (1957)
11. Lundin, P. M., and Holmdahl, S., *Acta Endocrinol.*, **26**, 388–94 (1957)
12. Emmens, C. W., Claringbold, P. J., and Lamond, D. R., *Nature*, **180**, 38–39 (1957)
13. Claringbold, P. J., and Lamond, D. R., *J. Endocrinol.*, **16**, 86–97 (1957–58)
14. Lamond, D. R., and Claringbold, P. J., *J. Endocrinol.*, **16**, 298–303 (1957–58)
15. Apostolakis, M., and Voigt, K. D., *Acta Endocrinol.*, **28**, 54–68 (1958)
16. Rosemberg, E., Smith, F., and Dorfman, R. I., *Endocrinology*, **61**, 337–40 (1957)

17. Pugsley, L. I., *Can. J. Biochem. and Physiol.*, **35**, 889-96 (1957)
18. Parlow, A. F., *Federation Proc.*, **17**, 402 (1958)
19. Noach, E. L., and van Rees, G. P., *Acta Endocrinol.*, **27**, 502-8 (1958)
20. Segal, S. J., *Science*, **126**, 1242-43 (1957)
21. Adams, D. D., and Purves, H. D., *Can. J. Biochem. and Physiol.*, **35**, 993-1004 (1957)
22. Bakke, J. L., Heideman, M. L., Jr., Lawrence, N. L., and Wiberg, C., *Endocrinology*, **61**, 352-67 (1957)
23. Squire, P. G., and Li, C. H., *Science*, **127**, 32 (1958)
24. Raake, I. D., Lostroh, A. J., Boda, J. M., and Li, C. H., *Acta Endocrinol.*, **26**, 377-87 (1957)
25. Buchholz, R., *Geburtsh. & Frauenh.*, **17**, 707-16 (1957)
26. Butt, W. R., Crooke, A. C., Ingram, J. D., and Round, B. P., *J. Endocrinol.*, **16**, 107-13 (1957-58)
27. Fletcher, R. F., and Brown, P. S., *Clin. Sci.*, **16**, 669-76 (1957)
28. Walter, K., *J. Endocrinol.*, **15**, 119-25 (1957)
29. Hamburger, C., and Johnsen, S. G., *Acta Endocrinol.*, **26**, 1-29 (1957)
30. Payne, R. W., Cahill, C., and Shetlar, M. R., *Federation Proc.*, **17**, 402 (1958)
31. Drèze, A., and Wodon, C., *Arch. intern. physiol.*, **62**, 559-60 (1954); *Chem. Abstr.*, **52**, 1401 (1958)
32. Bourrillon, R., Gut, R., Bouguereau, J., and Marcy, R., *Bull. soc. chim. biol.*, **39**, 1119-28 (1957)
33. van Wangenen, G., and Simpson, M. E., *Endocrinology*, **61**, 316-18 (1957)
34. Taber, E., Claytor, M., Knight, J., Gambrell, D., Flowers, J., and Ayers, C., *Endocrinology*, **62**, 84-89 (1958)
35. Henry, S. S., and van Dyke, H. B., *J. Endocrinol.*, **16**, 310-25 (1957-58)
36. Cole, H. H., Hamburger, C., and Neimann-Sørensen, A., *Acta Endocrinol.*, **26**, 286-98 (1957)
37. Antoniadis, H. N., Pennell, R. B., McArthur, J. W., Ingersoll, F. M., Ulfelder, H., and Oncley, J. L., *J. Biol. Chem.*, **228**, 863-74 (1957)
38. Li, C. H., and Coval, M., *J. Biol. Chem.*, **229**, 153-56 (1957)
39. Li, C. H., *J. Biol. Chem.*, **229**, 157-63 (1957)
40. Hadfield, G., *Lancet*, **I**, 1058-61 (1957)
41. Hadfield, G., and Young, S., *Lancet*, **I**, 568-69 (1958)
42. Pickford, G. E., and Kosto, B., *Endocrinology*, **61**, 177-96 (1957)
43. Bates, R. W., *Federation Proc.*, **17**, 187 (1958)
44. Hilliard, J., and West, P. M., *Endocrinology*, **60**, 797-801 (1957)
45. Carsten, M. E., and Pierce, J. G., *J. Biol. Chem.*, **229**, 61-67 (1957)
46. Sosenberg, M., and Money, W. L., *Endocrinology*, **61**, 12-19 (1957)
47. Brunish, R., *Endocrinology*, **62**, 437-41 (1958)
48. Raben, M. S., *Science*, **125**, 883-84 (1957)
49. Li, C. H., *Cancer*, **10**, 698-703 (1957)
50. Gemzell, C. A., and Li, C. H., *J. Clin. Endocrinol. and Metabolism*, **18**, 149-57 (1958)
51. Beck, J. C., McGarry, E. E., Dyrenfurth, I., and Venning, E. H., *Science*, **125**, 884-85 (1957)
52. Knobil, E., Morse, A., Wolf, R. C., and Greep, R. O., *Endocrinology*, **62**, 348-54 (1958)
53. Heijkenskjöld, F., and Gemzell, C. A., *Acta Endocrinol.*, **27**, 499-501 (1958)
54. Papkoff, H., and Li, C. H., *J. Biol. Chem.*, **231**, 367-77 (1958)

55. Dedman, M. L., Farmer, T. H., and Morris, C. J. O. R., *Biochem. J.*, **66**, 166-77 (1957)
56. Solem, J. H., Holtermann, H., and Skogrand, A., *Lancet*, **I**, 414-15 (1958)
57. Loringz, A. L., and Lancaster, G., *Science*, **126**, 124-25 (1957)
58. Sayers, G., Redgate, E. S., and Royce, P. C., *Ann. Rev. Physiol.*, **20**, 243-74 (1958)
59. Schreiber, V., *Endokrinologie*, **33**, 259-71 (1956)
60. Slusher, M. A., and Roberts, S., *Endocrinology*, **55**, 245-54 (1954)
61. Porter, J. C., and Rumsfeld, H. W., *Endocrinology*, **58**, 359-64 (1956)
62. Martini, L., and Morpurgo, C., *Nature*, **175**, 1127-28 (1955)
63. Sobel, H., Levy, R. S., Marmorston, J., Schapiro, S., and Rosenfeld, S., *Proc. Soc. Exptl. Biol. Med.*, **89**, 10-13 (1955)
64. McCann, S. M., *Endocrinology*, **60**, 664-76 (1957)
65. Guillemin, R., Hearn, W. R., Cheek, W. R., and Housholder, D. E., *Endocrinology*, **60**, 488-506 (1957)
66. Saffran, M., Schally, A. V., and Benfey, B. G., *Endocrinology*, **57**, 439-44 (1955)
67. Fuche, J., and Kahlson, G., *Acta Physiol. Scand.*, **39**, 327-47 (1957)
68. McCann, S. M., and Fruit, A., *Proc. Soc. Exptl. Biol. Med.*, **96**, 566-67 (1957)
69. Casentini, S., de Poli, A., and Martini, L., *Brit. J. Pharmacol.*, **12**, 166-70 (1957)
70. Leeman, S. E., and Munson, P. L., *Federation Proc.*, **17**, 387 (1958)
71. Nagareda, C. S., and Gaunt, R., *Endocrinology*, **48**, 560-67 (1951)
72. McDonald, R. K., Wagner, H. N., Jr., and Weise, V. K., *Proc. Soc. Exptl. Biol. Med.*, **96**, 652-55 (1957)
73. Schapiro, S., Marmorston, J., and Sobel, H., *Endocrinology*, **62**, 278-82 (1958)
74. Royce, P. C., and Sayers, G., *Federation Proc.*, **17**, 136 (1958)
75. de Wied, D., *Acta Endocrinol.*, **24**, 200-8 (1957)
76. de Wied, D., Bouman, P. R., and Smelik, P. G., *Endocrinology*, **62**, 605-13 (1958)
77. Curri, S., and Paoletti, R., *Boll. soc. ital. biol. sper.*, **32**, 1415-19 (1957)
78. Clayton, G. W., Bell, W. R., and Guillemin, R., *Proc. Soc. Exptl. Biol. Med.*, **96**, 777-79 (1957)
79. Schally, A. W., Saffran, M., and Zimmermann, B. U., *Biochem. J.*, **70**, 97-103 (1958)
80. Segal, M., *Rev. can. biol.*, **16**, 513-14 (1957) (abstract); Saffran, M., Schally, A. V., Segal, M., and Zimmermann, B. U., in *Zweites Internationales Symposium über Neurosekretion*, Lund, 55-59 (Bargmann, W., Hanström, B., Scharer, B., and Scharer, E., Eds., Springer, Berlin, Germany, 126 pp., 1958)
81. Rochefort, G. J., Rosenberger, J., and Saffran, M., *Rev. can. biol.*, **16**, 509-10 (1957) (abstract); *J. Physiol. (London)* (In press, 1959)
82. Hokin, M. R., Hokin, L. E., Saffran, M., Schally, A. V., and Zimmerman, B. U., *J. Biol. Chem.*, **233**, 811-13 (1958)
83. Landsmeer, J. M. F., *Acta Anat.*, **12**, 82-109 (1951)
84. Jewell, P. A., *J. Endocrinol.*, **14**, xxiv (1956) (abstract)
85. Fortier, C., and Ward, D. N., *Can. J. Biochem. and Physiol.*, **36**, 111-18 (1958)
86. Fortier, C., *Texas Repts. Biol. and Med.*, **16**, 68-78 (1958)
87. Barrett, A. M., and Sayers, G., *Endocrinology*, **62**, 637-45 (1958)
88. Saffran, M., *Can. J. Biochem. and Physiol.* (In press, 1959)
89. Birmingham, M. K., and Kurlents, E., *Endocrinology*, **62**, 47-60 (1958)
90. Eskine, I. A., *Ann. endocrinol. (Paris)*, **18**, 343-53 (1957)

91. Egdahl, R. H., Story, J. L., and Melby, J. C., *Federation Proc.*, **17**, 435 (1958)
92. Schmid, R., Gonzalo, L., Blobel, R., Muschke, E., and Tonutti, E., *Endokrinologie*, **34**, 65-91 (1957)
93. Bouman, P. R., Gaarenstroom, J. H., Smelik, P. G., and de Wied, D., *Acta Physiol. et Pharmacol. Neerl.*, **6**, 368-78 (1957)
94. Anderson, E., Bates, R. W., Hawthorne, E., Haymaker, W., Knowlton, K., Rioch, D. M., Spence, W. T., and Wilson, H., *Recent Progr. in Hormone Research*, **13**, 21-66 (1957)
95. Robinson, R., *Nature*, **181**, 484 (1958)
96. Sevy, R. W., Ohler, E. A., and Weiner, A., *Endocrinology*, **61**, 45-51 (1957)
97. McDonald, R. K., Evans, F. T., Weise, V. K., and Patrick, R. W., *Federation Proc.*, **17**, 109 (1958)
98. Schapiro, S., Marmorston, J., and Sobel, H., *Am. J. Physiol.*, **192**, 58-62 (1958)
99. Langecker, H., and Lurie, R., *Acta Endocrinol.*, **25**, 54-58 (1957)
100. Mialhe-Voloss, C., *Acta Endocrinol.*, **28**, Suppl. 35, 96 pp. (1958)
101. Kitay, J., Holub, D. A., and Jailer, J. W., *Proc. Soc. Exptl. Biol. Med.*, **97**, 165-69 (1958)
102. Long, C. N. H., and Bonnycastle, M. F. M., *Can. J. Biochem. and Physiol.*, **35**, 929-33 (1957)
103. Vivien, J. H., and Schott, L., *Compt. rend.*, **244**, 1263-65 (1957)
104. Nikitovitch-Winer, M., and Everett, J. W., *Endocrinology*, **62**, 522-32 (1958)
105. Van Dyke, D. C., Simpson, M. E., Lepovsky, J., Koneff, A. A., and Brobeck, J. R., *Proc. Soc. Exptl. Biol. Med.*, **95**, 1-5 (1957)
106. Bogdanove, E. M., *Endocrinology*, **60**, 689-97 (1957)
107. Robison, B. L., and Sawyer, C. H., *The Physiologist*, **1**, 72 (1957) (abstract)
108. Herbert, J., and Zuckerman, S., *Nature*, **180**, 547-48 (1957)
109. Barraclough, C. A., and Sawyer, C. H., *Endocrinology*, **61**, 341-51 (1957)
110. Gitsch, E., and Everett, J. W., *Endocrinology*, **62**, 400-9 (1958)
111. Moore, W. W., *Federation Proc.*, **17**, 113 (1958)
112. Nowell, N. W., and Chester Jones, I., *Acta Endocrinol.*, **26**, 273-85 (1957)
113. Armstrong, D. T., and Hansel, W., *Federation Proc.*, **17**, 6 (1958)
114. McCann, S. M., Mack, R., and Gale, C., *Federation Proc.*, **17**, 107 (1958)
115. Meites, J., *Proc. Soc. Exptl. Biol. Med.*, **96**, 728-30 (1957)
116. Flerkő, B., and Szentágothai, J., *Acta Endocrinol.*, **26**, 121-27 (1957)
117. Rothchild, I., *J. Clin. Endocrinol. and Metabolism*, **17**, 754-59 (1957)
118. Gans, E., and de Jongh, S. E., *Acta Physiol. et Pharmacol. Neerl.*, **5**, 271-77 (1957)
119. Goldman, J. N., Epstein, J. A., and Kupperman, H. S., *Endocrinology*, **61**, 166-72 (1957)
120. Smith, R. A., and Albert, A., *Proc. Staff Meetings Mayo Clinic*, **32**, 340-42 (1957)
121. Bogdanove, E. M., and Bogdanove, J. N., *Endocrinology*, **61**, 52-58 (1957)
122. D'Angelo, S., *Federation Proc.*, **17**, 32 (1958)
123. Knigge, K. M., and Bierman, S. M., *Am. J. Physiol.*, **192**, 625-30 (1958)
124. Samel, M., *Science*, **181**, 845-46 (1958)
125. Florsheim, W. H., Imagawa, D. T., and Greer, M. A., *Proc. Soc. Exptl. Biol. Med.*, **95**, 664-66 (1957)
126. Dorfman, R. I., *Cancer*, **10**, 741-45 (1957)
127. Pincus, G., *Bull. N. Y. Acad. Med.*, **33**, 587-98 (1957)
128. Bloch, K., *Vitamins and Hormones*, **15**, 119-50 (1957)

129. Wright, L. D., and Cleland, M., *Proc. Soc. Exptl. Biol. Med.*, **96**, 219-24 (1957)
130. Dituri, F., Rabinowitz, J. L., Hullin, R. P., and Gurin, S., *J. Biol. Chem.*, **229**, 825-36 (1958)
131. Kurath, P., Ganis, F. M., and Radakovitch, M., *J. Am. Chem. Soc.*, **79**, 5323-24 (1957)
132. Werbin, H., Chaikoff, I. L., and Jones, E., *Federation Proc.*, **17**, 333 (1958)
133. Frederickson, D. S., Peterson, R. E., and Steinberg, D., *Science*, **127**, 704-05 (1958)
134. Webb, J. L., and Heard, R. D. H., *Rev. can. biol.*, **16**, 523 (1957) (abstract)
135. Berliner, M. L., Berliner, D. L., and Dougherty, T. F., *J. Clin. Endocrinol. and Metabolism*, **18**, 109-14 (1958)
136. Chen, P. S., Jr., Schedl, H. P., Rosenfeld, G., and Bartter, F. C., *Proc. Soc. Exptl. Biol. Med.*, **97**, 683-85 (1958)
137. Stachenko, J. L. M., and Giroud, C. J. P., *Rev. can. biol.* **16**, 517-18 (1957) (abstract)
138. Eichhorn, J., and Hechter, O., *Proc. Soc. Exptl. Biol. Med.*, **97**, 614-19 (1958)
139. Eichhorn, J., and Hechter, O., *Proc. Soc. Exptl. Biol. Med.*, **95**, 311-15 (1957)
140. Marshall, C. W., Ralls, J. W., Saunders, F. J., and Riegel, B., *J. Biol. Chem.*, **228**, 339-48 (1957)
141. Grant, J. K., Symington, T., and Duguid, W. P., *J. Clin. Endocrinol. and Metabolism*, **17**, 933-44 (1957)
142. Tomkins, M., Curran, J. F., and Michael, P. J., *Federation Proc.*, **17**, 323 (1958)
143. Tomkins, M., and Michael, P. J., *Nature*, **180**, 337 (1957)
144. Hayano, M., Gut, M., and Peterson, D. H., *Federation Proc.*, **17**, 239 (1958)
145. Meyer, A. S., *Acta Endocrinol.*, **25**, 377-89 (1957)
146. Talalay, P., *Physiol. Revs.*, **73**, 362-87 (1957)
147. Hirsch, H. S., Berliner, D. L., and Samuels, L. T., *Arch. Biochem. Biophys.*, **71**, 91-99 (1957)
148. Nichols, J., Lescure, O. L., and Migeon, C. J., *J. Clin. Endocrinol. and Metabolism*, **18**, 444-52 (1958)
149. West, C. D., Damast, B., and Pearson, O. H., *J. Clin. Endocrinol. and Metabolism*, **18**, 15-27 (1958)
150. Bush, I. E., Swyer, G. I. M., Stern, H. I., and Willoughby, M. L. N., *J. Endocrinol.* **15**, 430-39 (1957)
151. Salhanik, H. A., and Berliner, D. L., *J. Biol. Chem.*, **227**, 583-90 (1957)
152. Wallach, S., Brown, H., Englert, E., and Eik-Nes, K., *J. Clin. Endocrinol. and Metabolism*, **17**, 945-58 (1957)
153. Snaith, A. H., *J. Clin. Endocrinol. and Metabolism*, **18**, 318-22 (1958)
154. Wolf, E. T., Mills, L. C., Newton, B. L., Tuttle, L. L. D., Hettig, R. A., Collins, V. P., and Gordon, W. B., *J. Clin. Endocrinol. and Metabolism*, **18**, 310-17 (1958)
155. Touchstone, J. C., *Federation Proc.* **17**, 323 (1958)
- 155a. Peterson, R. E., *J. Clin. Endocrinol. and Metabolism*, **17**, 1150-57 (1957)
156. Grant, J. K., Forrest, A. P. M., and Symington, T., *Acta Endocrinol.*, **26**, 195-203 (1957)
157. Wilson, H., Borris, J. J., and Bahn, R. C., *Endocrinology*, **62**, 135-49 (1958)
158. Cohen, A. I., Bloch, E., and Celozzi, E., *Proc. Soc. Exptl. Biol. Med.*, **95**, 304-09 (1957)
159. Reif, A. E., and Longwell, B. B., *Endocrinology*, **62**, 573-86 (1958)
160. Holzbauer, M., and Vogt, M., *J. Physiol.*, **138**, 449-59 (1957)

161. Holzbauer, M., *J. Physiol.*, **139**, 294-305 (1957)
162. Schönbaum, E., Large, R. E., Davidson, M., and Casselman, W. G. B., *Federation Proc.*, **17**, 306 (1958)
163. Cooper, D. Y., Kasparow, M., Blakemore, W. S., and Rosenthal, O., *Federation Proc.*, **17**, 205 (1958)
164. Hyde, P. M., and Williams, R. H., *J. Biol. Chem.*, **227**, 1063-82 (1957)
165. Collins, E. J., Forist, A. A., and Nadolski, E. B., *Proc. Soc. Exptl. Biol. Med.*, **97**, 558-60 (1958)
166. Smith, C. C., *J. Invest. Dermatol.*, **25**, 67-69 (1955)
167. Schwartz, R. D., Cohn, G. L., Bondy, P. K., Brodoff, M., Upton, G. U., and Spiro, H. M., *Proc. Soc. Exptl. Biol. Med.*, **97**, 648-50 (1958)
168. Tschan, D. N., and Adoni, L., *J. Invest. Dermatol.*, **28**, 385-86 (1957)
169. Daughaday, W. H., *J. Clin. Invest.*, **37**, 511-18 (1958)
170. Antoniadis, H. N., Pennell, R. B., Slaunwhite, W. R., Jr., and Sandberg, A. A., *J. Biol. Chem.*, **229**, 1071-79 (1958)
171. Daughaday, W. H., *J. Clin. Invest.*, **37**, 519-23 (1958)
172. Sandberg, A. A., and Slaunwhite, W. R., Jr., *J. Clin. Endocrinol. and Metabolism*, **17**, 1040-50 (1957)
173. Erlanger, B. F., Borek, F., Beiser, S. M., and Lieberman, S., *J. Biol. Chem.*, **228**, 713-28 (1957)
174. Peterson, R. E., Pierce, C. E., Wyngaarden, J. B., Bunim, J. J., and Brodie, B. B., *J. Clin. Invest.*, **36**, 1301-12 (1957)
175. Kuipers, F., Ely, R. S., and Kelley, V. C., *Endocrinology*, **62**, 64-74 (1958)
176. Wallace, E. Z., Silverberg, H. I., and Carter, A. C., *Proc. Soc. Exptl. Biol. Med.*, **95**, 805-08 (1957)
177. Englert, E., Brown, H., Wallach, S., and Simons, E. L., *J. Clin. Endocrinol. and Metabolism*, **17**, 1395-1406 (1957)
178. Hechter, O., Frank, E., Caspi, E., and Frank, H., *Endocrinology*, **60**, 705-10 (1957)
179. Anonymous, *Brit. Med. J.*, **I**, 270-72 (1958)
180. Brown, H., Englert, E., Wallach, S., and Simons, E. L., *J. Clin. Endocrinol. and Metabolism*, **17**, 1191-1201 (1957)
181. Tyler, F. H., *Am. J. Clin. Nutrition*, **5**, 377-86 (1957)
182. Tamm, J., Beckmann, I., and Voigt, K. D., *Acta Endocrinol.*, **27**, 292-302 (1958)
183. Brown, J. H. U., Anason, A., and Jacobs, J., *Am. J. Physiol.*, **190**, 259-64 (1957)
184. Bush, I. E., and Willoughby, M., *Biochem. J.*, **67**, 689-700 (1957)
185. Caspi, E., and Pechet, M. M., *Arch. Biochem. Biophys.*, **68**, 236-37 (1957)
186. Caspi, E., and Pechet, M. M., *J. Biol. Chem.*, **230**, 843-51 (1958)
187. Peterson, R. E., and Schmid, R., *J. Clin. Endocrinol. and Metabolism*, **17**, 1485-88 (1957)
188. Corte, G., and Johnson, W., *Proc. Soc. Exptl. Biol. Med.*, **97**, 751-55 (1958)
189. Kuipers, F., Ely, R. S., Hughes, E. R., and Kelley, V. C., *Proc. Soc. Exptl. Biol. Med.*, **95**, 187-89 (1957)
190. Brown, J. H. U., and Anason, A., *Endocrinology*, **62**, 103-7 (1958)
191. Glenn, E. M., Stafford, R. O., Lyster, S. C., and Bowman, B. J., *Endocrinology*, **61**, 128-42 (1957)
192. Dyrenfurth, I., Sybulski, S., Notchio, V., Beck, J. C., and Venning, E. H., *J. Clin. Endocrinol. and Metabolism*, **18**, 391-408 (1958)
193. Baulieu, E. E., and Jayle, M. F., *Bull. soc. chim. biol.*, **39**, 37-57 (1957)

194. Schriefers, H., Korus, W., and Dirscherl, W., *Acta Endocrinol.*, **26**, 331-44 (1957)
195. Bush, I. E., and Mahesh, V. B., *Biochem. J.*, **66**, 56P-57P (1957) (abstract)
196. Cope, C. L., and Black, E. G., *Clin. Sci.*, **17**, 147-63 (1958)
197. Fukushima, D. K., and Gallagher, T. F., *J. Biol. Chem.*, **226**, 725-33 (1957)
198. Weichselbaum, T. E., Elman, R., and Margraf, H. W., *J. Clin. Endocrinol. and Metabolism*, **17**, 1158-67 (1957)
199. Recknagel, R. O., *J. Biol. Chem.*, **227**, 273-84 (1957)
200. de Courcy, C., *J. Biol. Chem.*, **229**, 935-44 (1958)
201. Lombardo, M. E., and Hudson, P. B., *J. Biol. Chem.*, **229**, 181-88 (1957)
202. James, V. H. T., Baulieu, E. E., and Jayle, M. F., *Bull. soc. chim. biol.*, **39**, 59-64 (1957)
203. Vermeulen, A., *Lancet*, **II**, 79 (1957)
204. Borth, R., Linden, A., and Riondel, A., *Acta Endocrinol.*, **25**, 33-44 (1957)
205. Vestergaard, P., and Leverett, R., *Acta Endocrinol.*, **25**, 45-53 (1957)
206. Zicha, L., Scheiffarth, F., and Helfers, E., *Ärztliche Wochschr.*, **12**, 672-73 (1957)
207. Migeon, C. J., Keller, A. R., Lawrence, B., and Shepard, J. H., *J. Clin. Endocrinol. and Metabolism*, **17**, 1051-62 (1957)
208. Tamm, J., Beckmann, I., and Voiht, K. D., *Acta Endocrinol.*, **27**, 403-15 (1958)
209. Péron, F., and Dorfman, R. I., *Endocrinology*, **62**, 1-8 (1958)
210. Halkerston, I. D., Hillman, J., Palmer, D., Reiss, M., and Rundle, A., *J. Endocrinol.*, **16**, 156-63 (1957)
211. Brooks, R. V., and Prunty, F. T. G., *J. Endocrinol.*, **15**, 385-92 (1957)
212. Bradlow, H. L., and Gallagher, T. F., *J. Biol. Chem.*, **229**, 505-18 (1957)
213. Fotherby, K., Colas, A., Atherden, S. M., and Marrian, G. F., *Biochem. J.*, **66**, 664-69 (1957)
214. Crepy, O., Malassis, D., Meslin, F., and Jayle, M. F., *Acta Endocrinol.*, **26**, 43-56 (1957)
215. DeMeio, R. H., Lewycka, C., Wizerkaniuk, M., and Salciunas, O., *Biochem. J.*, **68**, 1-5 (1958)
216. Robbins, P. W., and Lipmann, F., *J. Biol. Chem.*, **229**, 837-51 (1958)
217. Roy, A. B., *Biochem. J.*, **66**, 700-07 (1957)
218. Eik-Nes, K., Demetriou, J. A., Mayne, Y. C., and Jones, R. S., *Proc. Soc. Exptl. Biol. Med.*, **96**, 409-11 (1957)
219. Sandberg, A. A., and Slaunwhite, W. R., Jr., *Proc. Soc. Exptl. Biol. Med.*, **96**, 658-62 (1957)
220. Lazo-Wasem, E. A., and Hier, S. W., *Endocrinology*, **62**, 308-12 (1958)
221. Kellie, A. E., and Smith, E. R., *Biochem. J.*, **66**, 490-95 (1957)
222. Kornel, L., *Lancet*, **II**, 775-76 (1957)
223. Englert, E., Brown, H., Willardson, D., Wallach, S., and Simons, E. L., *J. Clin. Endocrinol. and Metabolism*, **18**, 36-48 (1958)
224. Nabarro, J. D. N., and Moxham, A., *Lancet*, **II**, 624-25 (1957)
225. Klopfer, A., Strong, J. A., and Cook, L. R., *J. Endocrinol.*, **15**, 180-89 (1957)
226. Jaoudé, F. A., Baulieu, E. E., and Jayle, M. F., *Acta Endocrinol.*, **26**, 30-42 (1957)
227. Masouda, M., *J. Clin. Endocrinol. and Metabolism*, **17**, 1181-90 (1957)
228. Appel, W., *Ärztliche Wochschr.*, **12**, 156-59 (1957)
229. Martin, M. M., Gray, C. H., Livingstone, J. L., and Lunnnon, B. J., *J. Clin. Endocrinol. and Metabolism*, **17**, 1168-80 (1957)

230. Haydar, N. A., St. Marc, J. R., Reddy, W. J., Laidlaw, J. C., and Thorn, G. W., *J. Clin. Endocrinol. and Metabolism*, **18**, 121-33 (1958)
231. Mandelstram, P., Goldzieher, J. W., Sorooff, H. S., and Green, N., *J. Clin. Endocrinol. and Metabolism*, **18**, 284-99 (1958)
232. Sandberg, A. A., Eik-Nes, K., Migeon, C. J., and Koepf, G. F., *J. Lab. Clin. Med.*, **50**, 286-96 (1957)
233. Soffer, L. J., Geller, J., and Gabrilove, J. L., *J. Clin. Endocrinol. and Metabolism*, **17**, 878-83 (1957)
234. Jenkins, J. S., and Spence, A. W., *J. Clin. Endocrinol. and Metabolism*, **17**, 621-31 (1957)
235. Geller, J., Alvarez, A. S., Gutman, A., de Freetas, A., Gabrilove, J. L., and Soffer, L. J., *J. Clin. Endocrinol. and Metabolism*, **18**, 409-16 (1958)
236. Zondek, H., Zondek, G. W., and Leszynsky, H. E., *Acta Endocrinol.*, **26**, 91-95 (1957)
237. Bloom, A., *Postgrad. Med. J.*, **33**, 499-504 (1957)
238. Haynes, R. C., Jr., *Federation Proc.*, **17**, 376 (1958)
239. Koritz, S. B., and Péron, F. G., *J. Biol. Chem.*, **230**, 343-52 (1958)
240. Cohen, A. I., Bloch, E., and Celozzi, E., *Proc. Soc. Exptl. Biol. Med.*, **95**, 304-9 (1957)
241. Poore, W., and Hollander, V. P., *Proc. Soc. Exptl. Biol. Med.*, **97**, 644-46 (1958)
242. Hilton, J. G., Weaver, D. C., Muelheims, G., Glaviano, V. V., and Wégria, R., *Am. J. Physiol.*, **192**, 525-30 (1958)
243. Kline, I. T., *Endocrinology*, **61**, 85-92 (1957)
244. Clayton, B. E., and Hammant, J. E., *J. Endocrinol.*, **15**, 255-65 (1957)
245. Salomon, L., *Texas Repts. Biol. and Med.*, **15**, 934-39 (1957)
246. Dayton, P. G., and Burns, J. J., *J. Biol. Chem.*, **321**, 85-91 (1958)
247. Salomon, L., *Texas Repts. Biol. and Med.*, **16**, 137-52 (1958)
248. Salomon, L., *Texas Repts. Biol. and Med.*, **16**, 153-65 (1958)
249. Salomon, L., *Texas Repts. Biol. and Med.*, **15**, 925-33 (1957)
250. Slusher, M. A., and Roberts, S., *Endocrinology*, **61**, 98-105 (1957)
251. Briggs, F. N., and Toepel, W., *Endocrinology*, **62**, 24-29 (1958)
252. Salomon, L. L., *J. Biol. Chem.*, **228**, 163-70 (1957)
253. Guillemin, R., Clayton, G. W., Smith, J. D., and Lipscomb, H. S., *Compt. rend.*, **245**, 1834 (1957)
254. Guillemin, R., Clayton, G. W., Smith, J. D., and Lipscomb, H. S., *Federation Proc.*, **17**, 63 (1958)
255. Heroux, O., and Gridgeman, N. T., *Can. J. Biochem. and Physiol.*, **36**, 209-16 (1958)
256. Brown, K. I., Brown, D. J., and Meyer, R. K., *Am. J. Physiol.*, **192**, 43-50 (1958)
257. Koritz, S. B., Péron, F. G., and Dorfman, R. I., *J. Biol. Chem.*, **226**, 643-50 (1957)
258. Lostroh, A. J., *Proc. Soc. Exptl. Biol. Med.*, **97**, 828-32 (1958)
259. Lostroh, A. J., and Woodward, P., *Endocrinology*, **62**, 498-505 (1958)
260. Nicholls, D., and Graham, C., *Can. J. Biochem. and Physiol.*, **35**, 401-6 (1957)
261. Allen, J. M., *Endocrinology*, **61**, 368-74 (1957)
262. Kalant, O. J., and Sellers, E. A., *Endocrinology*, **61**, 264-70 (1957)
263. Kalant, O. J., *Endocrinology*, **62**, 237-44 (1958)
264. Glick, D., Freier, E. P., and Ochs, M. J., *J. Biol. Chem.*, **226**, 77-82 (1957)
265. Engel, F. L., *Am. J. Clin. Med.*, **5**, 417-30 (1957)

266. Engel, M. G., and Engel, F. L., *Endocrinology*, **62**, 75-77 (1958)
267. Engel, F. L., *Yale J. Biol. and Med.*, **30**, 201-23 (1957)
268. Higginbotham, R. D., and Dougherty, T. F., *Proc. Soc. Exptl. Biol. Med.*, **96**, 446-70 (1957)
269. Langley, L. L., and Gunthorpe, C. H., *Am. J. Physiol.*, **191**, 342-44 (1957)
270. Leonard, S. L., *Endocrinology*, **60**, 619-24 (1957)
271. Kostyo, J. L., *Proc. Soc. Exptl. Biol. Med.*, **97**, 405-7 (1958)
272. Froesch, E. R., Ashmore, J., and Renold, A. E., *Endocrinology*, **62**, 614-20 (1958)
273. Gambassi, G., and Maggi, V., *Boll. soc. ital. biol. sper.*, **32**, 1539-41 (1957)
274. Papper, S., Saxon, L., and Alpert, H. C., *J. Lab. Clin. Med.*, **50**, 384-90 (1957)
275. Demanet, J. C., de Meutter, R. C., and Gefits, W., *Acta Endocrinol.*, **27**, 281-91 (1958)
276. Altszuler, N., Steele, R., Wall, J. S., and de Bodo, R. C., *Am. J. Physiol.*, **191**, 29-33 (1957)
277. Altszuler, N., Steele, R., Wall, J. S., and de Bodo, R. C., *Am. J. Physiol.*, **192**, 219-26 (1958)
278. Ashmore, J., Cahill, G. F., Jr., Hillman, R., and Renold, A. E., *Endocrinology*, **62**, 621-26 (1958)
279. Rosen, F., Roberts, N. R., Budnick, L. E., and Nichol, C. A., *Science*, **127**, 287-88 (1958)
280. Froesch, E. R., Winegrad, A. I., Renold, A. E., and Thorn, G. W., *J. Clin. Invest.*, **37**, 524-32 (1958)
281. Buse, J., Gundersen, K., and Lukens, F. D. W., *Diabetes*, **6**, 428-32 (1957)
282. Whitney, J. E., and Young, F. G., *Biochem. J.*, **66**, 648-51 (1957)
283. Vallance-Owen, J., and Lukens, F. D. W., *Endocrinology*, **60**, 625-33 (1957)
284. Fabrykant, M., Jackson, R. S., and Ashe, B. I., *Metabolism, Clin. and Exptl.*, **6**, 116-26 (1957)
285. Denko, C. W., *J. Lab. Clin. Med.*, **51**, 174-77 (1958)
286. Lehr, D., Wajda, I., and Krukowski, M., *Federation Proc.*, **17**, 388 (1958)
287. Kowalewski, K., *Proc. Soc. Exptl. Biol. Med.*, **97**, 432-34 (1958)
288. Monkhouse, F. C., MacKneson, R. G., and Bammers, G., *Proc. Soc. Exptl. Biol. Med.*, **95**, 489-92 (1957)
289. Jackson, K. L., and Entenman, C., *Proc. Soc. Exptl. Biol. Med.*, **97**, 184-86 (1958)
290. Rupp, J. J., and Paschkis, K. E., *Proc. Soc. Exptl. Biol. Med.*, **95**, 477-79 (1957)
291. Dolowitz, D. A., Dougherty, T. F., Higginbotham, R. D., and McNeil, C., *Arch. Otolaryngol.*, **66**, 245-48 (1957)
292. Schultz, R. L., Okawaki, M. S., Seerveld, H. L., and Mullins, J. S., *Proc. Soc. Exptl. Biol. Med.*, **96**, 664-67 (1957)
293. Scheiffarth, F., Berg, G., Legler, F., and Schuler, E., *Arzneimittel-Forsch.*, **7**, 361-65 (1957)
294. Shewell, J., *Brit. J. Pharmacol.*, **12**, 133-39 (1957)
295. Noall, M. W., Riggs, T. R., Walker, L. M., and Christensen, H. N., *Science*, **126**, 1002-5 (1957)
296. Kivirikko, K. J., and Liesmaa, M., *Acta Endocrinol.*, **27**, 441-45 (1958)
297. Bach, S. J., Carter, S. B., and Killip, J. D., *Biochim. et Biophys. Acta*, **28**, 168-75 (1958)
298. Wolf, R. C., *Am. J. Physiol.*, **190**, 129-32 (1957)
299. Hudson, B., and Doig, A., *Australasian Ann. Med.*, **8**, 228-37 (1957)

300. Farrell, G. L., Fleming, R. B., Rauschkolb, E. W., Yatsu, F. M., McCally, M., and Anderson, C. H., *Endocrinology*, **62**, 506-12 (1958)
301. Newman, A. E., Redgate, E. S., Yatsu, F. M., and Farrell, G. L., *Federation Proc.*, **17**, 117 (1958)
302. Daily, W. J. R., and Ganong, W. F., *Endocrinology*, **62**, 442-54 (1958)
303. Giroud, C. J. P., Stachenko, J., and Piletta, P., in *An International Symposium on Aldosterone*, 56-72 (Muller, A. F., and O'Connor, C. M., Eds., Little, Brown and Company, Boston, Mass., 232 pp., 1958)
304. Orti, E., Ralli, E. P., Laken, B., and Dumm, M. E., *Am. J. Physiol.*, **191**, 323-28 (1957)
305. Eisenstein, A. B., and Hartroft, P. M., *Endocrinology*, **60**, 634-40 (1957)
306. Hartroft, P. M., and Eisenstein, A. B., *Endocrinology*, **60**, 641-51 (1957)
307. Race, G. J., Nickey, W. M., Wolf, P. S., and Jordon, E. J., *Arch. Pathol.*, **64**, 312-23 (1957)
308. Johnson, B. B., Lieberman, A. H., and Mulrow, P. J., *J. Clin. Invest.*, **36**, 757-66 (1957)
309. Anonymous, *Lancet*, **II**, 730-32 (1957)
310. Fine, D., Meiselas, L. E., and Auerbach, T., *J. Clin. Invest.*, **37**, 232-43 (1958)
311. Thomas, S., *J. Physiol. (London)*, **139**, 337-52 (1957)
312. Taymor, R. C., and Friedberg, C. K., *J. Appl. Physiol.*, **11**, 125-28 (1957)
313. Davis, J. O., Bahn, R. C., Goodkind, M. J., and Ball, W. C., Jr., *Am. J. Physiol.*, **191**, 329-38 (1957)
314. Davis, J. O., Pechet, M. M., Ball, W. C., Jr., and Goodkind, M. J., *J. Clin. Invest.*, **36**, 689-94 (1957)
315. Ball, W. C., Jr., and Davis, J. O., *Am. J. Physiol.*, **191**, 339-41 (1957)
316. Davis, J. O., and Ball, W. C., Jr., *Am. J. Physiol.*, **192**, 538-42 (1958)
317. Wolff, H. P., Koczorek, K. R., Jesch, W., and Buchborn, E., *Klin. Wochschr.*, **34**, 366-71 (1956)
318. Wolff, H. P., Koczorek, K. R., and Buchborn, E., *Lancet*, **II**, 63-66 (1957)
319. Wolff, H. P., Koczorek, K. R., and Buchborn, E., *Acta Endocrinol.*, **27**, 45-58 (1958)
320. Genest, J., Koiw, E., Nowaczynski, W., and Leboeuf, G., *Proc. Soc. Exptl. Biol. Med.*, **97**, 676-79 (1958)
321. Driscoll, T. E., Maultsby, M. M., Farrell, G. L., and Berne, R. H., *Am. J. Physiol.*, **191**, 140-44 (1957)
322. Little, B., Smith, O. W., Jessiman, A. G., Selenkow, H. A., van't Hoff, W., Eglin, J. M., and Moore, F. D., *J. Clin. Endocrinol. and Metabolism*, **18**, 425-43 (1958)
323. Gierd, R. J., and Green, D. M., *Acta Endocrinol.*, **28**, 1-10 (1958)
- 323a. Ferguson, F. P., Smith, D. C., and Barry, J. Q., *Endocrinology*, **60**, 761-67 (1957)
324. Share, L., and Stadler, J. B., *Endocrinology*, **62**, 119-28 (1958)
325. Dingman, J. F., *Am. J. Med. Sci.*, **235**, 79-99 (1958)
326. Richter, H. S., *Proc. Soc. Exptl. Biol. Med.*, **97**, 141-44 (1958)
327. Goldsmith, R. S., Bartter, F. C., Rosch, P. J., Meroney, W. H., and Herndon, E. G., Jr., *J. Clin. Endocrinol. and Metabolism*, **18**, 323-24 (1958)
328. Skanse, B., and Hökfelt, B., *Acta Endocrinol.*, **28**, 29-36 (1958)
329. Elron, D. H., *Acta Endocrinol.*, **26**, 209-14 (1957)
330. Friedman, S. M., Nakashima, M., and Friedman, C. L., *Endocrinology*, **62**, 259-67 (1958)

331. Cowie, A. T., and Tindal, J. S., *J. Endocrinol.*, **16**, 403-14 (1958)
332. Gross, F., and Lichtlen, P., in *An International Symposium on Aldosterone*, 39-49 (Muller, A. F., and O'Connor, C. M., Eds., Little, Brown & Co., Boston, Mass., 232 pp., 1958); *Chem. Abstr.*, **51**, 9741 (1957)
333. Valuera, T. K., *Fiziol. Zhur. Akad. Nauk. Ukr. R. S. R.*, **1**, 93-96 (1957)
334. Sherwood Jones, E., *Experientia*, **15**, 72 (1958)
335. Wilson, D. L., *Am. J. Physiol.*, **190**, 104-8 (1957)
336. Sweet, A. Y., Levitt, M. F., and Hodes, H. L., *J. Clin. Invest.*, **37**, 65-69 (1958)
337. Nicholson, T. F., *Can. J. Biochem. and Physiol.*, **35**, 641-44 (1957)
338. Kagawa, C. M., Cella, J. A., and Van Arman, C. G., *Science*, **126**, 1015-16 (1957)
339. Liddle, G. W., *Science*, **126**, 1016-18 (1957)
340. Lichtlen, P., *Acta Endocrinol.*, **27**, 1-35 (1958)
341. Raisz, L. G., McNeely, W. F., Saxon, L., and Rosenbaum, J. D., *J. Clin. Invest.*, **36**, 767-79 (1957)
342. Kovács, K., Kovács, B., Kovács, G. S., and Petri, G., *Endokrinologie*, **34**, 32-36 (1957)
343. Neher, R., *Clin. Chem.*, **3**, 344-70 (1957)
344. Soffer, L. J., Gutman, A., Geller, J., and Gabrilove, J. L., *Bull. N. Y. Acad. Med.*, **33**, 665-80 (1957)
345. Muller, A. F., and O'Conner, C. M., Eds., *An International Symposium on Aldosterone* (Little, Brown & Co., Boston, Mass., 232 pp., 1958)
346. Kumar, D., Hall, A. E. D., Nakashima, R., and Gornall, A. G., *Can. J. Biochem. and Physiol.*, **35**, 113-18 (1957)
347. Gross, F., Loustalot, P., and Meier, R., *Acta Endocrinol.*, **26**, 417-23 (1957)
348. Gornall, A. G., Grundy, H. M., and Koladich, C. J., *Rev. can. biol.*, **16**, 486 (1957) (abstract)
349. Tobian, L., and Redleaf, P. D., *Am. J. Physiol.*, **189**, 451-54 (1957)
350. Knowlton, A. I., and Loeb, E. N., *J. Clin. Invest.*, **36**, 1295-1300 (1957)
351. Knowlton, A. I., Loeb, E. N., and Stoerk, H. C., *Endocrinology*, **60**, 768-77 (1957)
352. Dunihue, F. W., and Robertson, W. van B., *Endocrinology*, **61**, 293-99 (1957)
353. Neff, A. W., and Correll, J. T., *Proc. Soc. Exptl. Biol. Med.*, **95**, 227-29 (1957)
354. Chappel, C. I., Charest, M. P., Cahill, J., and Grant, G. A., *Endocrinology*, **60**, 677-78 (1957)
355. Chappel, C. I., Cahill, J., Rona, G., and Grant, G. A., *Endocrinology*, **62**, 30-40 (1958)
356. Masson, G. M. C., Koritz, S. B., and Péron, F. G., *Endocrinology*, **62**, 229-33 (1958)
357. Skelton, F. R., *Endocrinology*, **62**, 365-68 (1958)
358. Masson, G. M. C., Corcoran, A. C., and Page, I. H., *Endocrinology*, **61**, 409-18 (1957)
359. Wolferth, C. C., Fitts, W. T., Jeffers, W. A., and Sellers, A. M., *Bull. N. Y. Acad. Med.*, **33**, 151-70 (1957)
360. Birke, G., Gemzell, C. A., Plantin, L. O., and Robbe, H., *Acta Endocrinol.*, **27**, 389-402 (1958)
361. Kawahara, H., *J. Clin. Endocrinol. and Metabolism*, **18**, 325-27 (1958)
362. Baulieu, E. E., de Vigan, M., Bricaire, H., and Jayle, M. F., *J. Clin. Endocrinol. and Metabolism*, **17**, 1478-82 (1957)

363. Herrmann, W., and Silverman, L., *J. Clin. Endocrinol. and Metabolism*, **17**, 1482-85 (1957)
364. Appleby, J. I., and Norymberski, J. K., *J. Endocrinol.*, **15**, 310-19 (1957)
365. Schüller, E., *Acta Endocrinol.*, **25**, 345-64 (1957)
366. Christianson, M., and Chester Jones, I., *J. Endocrinol.*, **15**, 17-42 (1957)
367. Martin, J. D., and Mills, I. H., *Clin. Sci.*, **17**, 137-46 (1958)
368. Migeon, C. J., Bertrand, J., and Wall, P. E., *J. Clin. Invest.*, **36**, 1350-62 (1957)
369. Little, B., Vance, V. K., and Rossi, E., *J. Clin. Endocrinol. and Metabolism*, **18**, 49-53 (1958)
370. McKay, E., Assali, N. S., and Henley, M., *Proc. Soc. Exptl. Biol. Med.*, **95**, 653-56 (1957)
371. Nowaczynski, W., Koiv, E., and Genest, J., *Can. J. Biochem. and Physiol.*, **35**, 425-43 (1957)
372. Laidlaw, J. C., Cohen, H., and Gornall, A. G., *J. Clin. Endocrinol. and Metabolism*, **18**, 222-25 (1958)
373. Peterson, R. E., *J. Clin. Invest.*, **37**, 736-43 (1958)
374. Jakobson, T., *Acta Endocrinol.*, **27**, 432-40 (1958)
375. Williams, G. A., Crispell, K. R., and Parson, W., *J. Clin. Endocrinol. and Metabolism*, **17**, 1347-53 (1957)
376. Brown, H., Englert, E., and Wallach, S., *J. Clin. Endocrinol. and Metabolism*, **18**, 167-79 (1958)
377. Aterman, K., *Endocrinology*, **60**, 711-17 (1957)
378. Lostroh, A. J., and Li, C. H., *Endocrinology*, **62**, 484-92 (1958)
379. Bois, P., and Selye, H., *J. Endocrinol.*, **15**, 171-79 (1957)
380. Roy, S. N., Karkun, J. N., and Sur, R. N., *Acta Endocrinol.*, **27**, 216-24 (1958)
381. Kowalewski, K., *Acta Endocrinol.*, **27**, 257-61 (1958)
382. Mikulay, L., and Nemeth, S., *J. Clin. Endocrinol. and Metabolism*, **18**, 539-42 (1958)
383. Brooks, R. V., Hetzel, B. S., McSwiney, R. R., and Prunty, F. T. G., *J. Endocrinol.*, **15**, 320-6 (1957)
384. Beck, R. N., *Endocrinology*, **62**, 9-14 (1958)
385. Bastenie, P. A., and Ermans, A. M., *Helv. Med. Acta*, **24**, 188-92 (1957)
386. Bastenie, P. A., and Ermans, A. M., *Endocrinology*, **62**, 245-50 (1958)
387. Knigge, K. M., Goodman, R. S., and Solomon, D. H., *Am. J. Physiol.*, **189**, 415-19 (1957)
388. Fremont-Smith, K., Iber, F. L., and Plough, I. C., *J. Clin. Invest.*, **36**, 1313-18 (1957)
389. Doisy, R. J., and Lardy, H. A., *Am. J. Physiol.*, **190**, 142-46 (1957)
390. Donhoffer, S., Varnai, I., and Sziebert-Horvath, E., *Nature*, **181**, 345-46 (1958)
391. Hetzel, B. S., Williams, R., and Lander, H., *Australasian Ann. Med.*, **6**, 218-27 (1957)

THE PANCREAS: INSULIN AND GLUCAGON¹

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Since the investigations on the external secretion of the pancreas are reviewed annually in the chapter on the digestive system, this chapter will be limited to the islands of Langerhans, insulin, and glucagon. Insulin and glucagon have been the subjects of several recent reviews in which the reader will find many references not included here.

THE ALPHA CELLS

The basic attributes of the alpha cells have been reviewed by Lazarow (1). He pointed out the differentiation of alpha cells from beta cells by their staining properties and the principal stains employed. Since staining is generally regarded as a satisfactory means of identification of alpha cells, some comments on the difficulties of such staining deserve attention. Thus, in a discussion of this topic Goldner & Volk (2) noted that with the Gomori stain, 26 per cent of the islet cells were identified as alpha cells whereas a silver impregnation method identified only 6 per cent as alpha cells. Ferner (3) adds to this comment the fact that silver stains appear satisfactory in man but not in the rat. Lazarow (1) and Fodden (4) cite evidence that staining methods also permit the probable distinction between alpha cells and the argentaffin cells of the gastrointestinal tract. Alpha and beta cells are also differentiated by their response to selectively toxic agents. This refers particularly to the damaging effect of cobalt, synthalin, or *p*-aminobenzene-sulfonamidoisopropylthiadiazole (IPTS) (3), and to the selective action of alloxan, dehydroascorbic acid, and other agents on the beta cells. The meaning of cobalt poisoning requires further study since Fodden (5) found no damage of any significance from cobaltous chloride in the islands of the monkey, dog, cat, rabbit, rat, and teleost fish. Finally, Lazarow (1) recalls the selective damage to the beta cells in experimental diabetes in dogs and cats, in which the alpha cells appear intact. Under electron microscopy (6) the alpha cells differ from the beta cells in several respects. Thus, the concentration of the granules of the alpha cells is greater than that of the beta granules; the Golgi complex is smaller, and the amount of ergastoplasm is less than in the beta cells. The finding of Bencosme *et al.* (7) that the uncinate process of the dog's pancreas is devoid of alpha cells, whereas the beta cells are present, may well provide a better method of studying alpha cell function than the use of toxins such as cobalt.

The influence of hypophysectomy and pituitary hormones on the islets.—The morphological changes in the rat have been described by Goldner & Volk (2) and Mosca (8). There was no change in the appearance or number of alpha

¹ The survey of the literature on which this review is based was concluded in May, 1958.

and beta cells after hypophysectomy in adult rats. Mosca (8) adds observations in young rats. In the first two months of life the alpha:beta cell ratio was 35:65; in adult rats it was 25:75. In young hypophysectomized rats the alpha:beta cell ratio remained at the high juvenile level with advancing age. The combined administration of growth hormone and corticotropin brought the cells to the adult proportions but growth hormone alone failed to do so. In contrast, in normal rats observed after colchicine, growth hormone increased the number of mitoses in the beta cells. Corticotropin diminished the number of mitoses in both alpha and beta cells. Changes in the appearance of the islands have been related to the duration of treatment with growth hormone (2). At three weeks of such treatment there was an increase in alpha cells and island hypertrophy. At six weeks, the alpha cells decreased and both alpha and beta cells were degranulated (2). In contrast, Kracht (9) found no histologic evidence of any direct effect of somatotropin on the alpha cells, but on the basis of the involution of these cells after hypophysectomy he assumes an influence of the pituitary on the alpha cells. In spite of the diabetogenic effect of certain pituitary extracts there still seems to be no clear evidence of a pancreatotrophic hormone (10).

GLUCAGON, THE HYPERGLYCEMIC GLYCOGENOLYTIC FACTOR (HGF)

The history of glucagon from 1923 to the present has been reviewed (11, 12, 13). Schulze's (14) review of Burger's work is particularly noted for its historical interest.

Chemical nature of glucagon.—The purification, crystallization, and amino acid content, reported in 1953, have been further described (15, 16) and reviewed (17). The same group of workers has examined the lack of effect of glucagon on the mouse assay of insulin (18) and has shown that crystalline insulin contains 0.3 to 0.5 per cent of glucagon (19). The extraction, chemical identification, and reasonably quantitative assay of this substance derived from pancreas appear to be well advanced. However, when such assay methods are applied to other extracts obtained under various physiological situations the problem becomes confused. Thus, in dogs treated with growth hormone, a hyperglycemic substance was liberated into the pancreaticoduodenal vein (20). Transfusion of pancreaticoduodenal vein blood from these animals caused hyperglycemia in depancreatized dogs. This effect (a) was produced after the administration of growth hormone to depancreatized dogs whose pancreaticoduodenal vein no longer drained a pancreas and (b) was abolished by treatment of the recipient dog with dihydroergotamine, which did not prevent the rise of blood sugar after the administration of glucagon. Likewise, the urinary extract studied by Moya (21) was both hyperglycemic and glycogenolytic but probably was not glucagon. In confirming the influence of glucagon on the reactivation of phosphorylase in rabbit liver, Cornblath (22) used the liberation of inorganic phosphorus by liver homogenates as a measure of this effect. Epinephrine, glucagon, and ephedrine each increased phosphorylase activity. Ephedrine and glucagon were additive in this effect whereas ephedrine and epinephrine were not. Mohnike & Boser (23, 24) have prepared a pancreas nucleoprotein identical

with phosphorylase (25), which is glycogenolytic. They conclude that there are two glycogenolytic substances in the pancreas: glucagon, which acts by influencing the activity of liver phosphorylase (26), and a pancreatic phosphorylase which is likewise glycogenolytic. To these must be added epinephrine or similar substances. As Foa *et al.* (13) state, "the chemical characteristics of glucagon demonstrate that this substance is entirely different from the hyperglycemic nucleoprotein described by Mohnike and Boser" (23, 24) and from other materials which he cites. Yet the physiologist is still baffled by the fact that so many agents produce hyperglycemia and glycogenolysis.

Site or sites of origin of glucagon.—The extraction of glucagon from the pancreas and glucagonlike materials from the mucosa of the upper gastrointestinal tract (13) and other tissues (27) is known. There are facts and discussions for and against the hypothesis that the alpha cells are the source of glucagon. The word "source" may mean the source of an extract of the tissue or the source of secretion of the substance. The results of the extraction of tissues to yield a hyperglycemic substance will first be noted. As stated above there have been many reports of active, i.e. hyperglycemic, extracts, but accurate identification and quantification of these have not yet been developed. The usual assay methods have been reviewed (13) and a recent modification has been proposed (28). A concise, documented review of the present situation is given by Foa *et al.* (13) and may be illustrated by selected reports. Thus, extracts from the pancreas of cobalt-treated dogs have been prepared (29). Even when almost total absence of alpha cells was found histologically, the extracts were hyperglycemic. In addition, hyperglycemic extracts were obtained from a cobalt-treated, alloxan diabetic dog and from animals in which the exocrine portion of the pancreas had been destroyed by duct ligation or ethionine. Hyperglycemic extracts have been obtained from the endocrine pancreatic nodules of fish (30). In contrast, Vuylsteke *et al.* (31) found significant reduction or even absence of glucagon in the extracts of cobalt-treated guinea pigs, and Bencosme *et al.* (32) obtained no glucagon from those portions of the dog's pancreas which are naturally devoid of alpha cells. Substances which are glucagonlike in that they are hyperglycemic have been extracted from many tissues (27) but have not been identified as glucagon. In summary, it seems that the alpha cells of the islands of Langerhans are the most probable source of glucagon but that the question is still unsettled.

The hormonal nature and secretion of glucagon.—This has been so well reviewed (13, 33) that only a few points will be noted here. There is no known or proven syndrome of glucagon-deficiency. The hypoplasia of alpha cells reported (34, 35) to occur in certain infants with hypoglycemia has not been established as a glucagon deficiency state nor relieved by glucagon administration. The claim that the reduced insulin requirement of depancreatized animals (i.e., reduced in comparison to the requirement in alloxan diabetes) results from glucagon deficiency is tenuous if not unwarranted. (a) While fasting the alloxan diabetic animal's disease is less severe (36). (b) In at least one of the experiments in which the depancreatized animal's weight was well

maintained by pancreatic enzymes, the authors (37) concluded: "Removal of the pancreas from a pituitary-diabetic dog resulted in a slight and possibly not significant fall in insulin requirement." (c) The relation of fat absorption to insulin requirement has been largely disregarded and may be of major significance as judged by the report of Singh (38) that severe diabetics needed only one-half to one-fourth of their usual dose of insulin when dietary fat was greatly reduced.

In spite of several trials, documented by Foa *et al.* (13) and Elrick *et al.* (39), the one significant glucagon-excess syndrome is the prolonged hyperglycemia produced in rats by the daily administration of large doses of glucagon in oil (40). At present the mechanism and meaning of this require further study.

The question whether or not glucagon is secreted has perhaps best been investigated and reviewed by Foa *et al.* (13). Their cross-circulation experiments demonstrated a hyperglycemic effect when blood from the pancreatic vein of a donor dog was perfused into a recipient (normal) dog under certain conditions. (a) When the normal dog was treated with insulin and was hypoglycemic the blood sugar of the recipient increased. In control experiments, in which a mesenteric vein was used instead of the pancreatic vein, the blood sugar of the recipient decreased. (b) When glucose or glucagon was injected into the donor dog elevating its blood sugar concentration, hypoglycemia occurred in the recipient, possibly because of a suppression of glucagon in the pancreatic venous blood of the donor and, one might add, possibly because of increased insulin secretion evoked by glucagon hyperglycemia. Other experiments of this sort are reviewed. As Elrick (33) says, the identity of the substance in the pancreatic vein remains in doubt in view of the report from Best's (20) laboratory, to which one may add the possibility that the "phosphorylase" extracted from the gland (24) might also enter the vein.

Glucagon loses its activity when perfused through the liver of the normal animal (41, 42) but not of the diabetic animal (42). Here, as in its destruction by various tissue extracts, it appears to compete with the enzyme(s) which destroy insulin (43, 44).

Effects of glucagon.—The most conspicuous effects of the administration of pure glucagon are a rise in the concentration of blood glucose and an immediate decrease in liver glycogen. The rise in the level of blood glucose is characterized by its rapid onset in 10 to 30 min. after intravenous administration and by the effectiveness of a very small dose. Thus, a cat unit of glucagon has been defined as the quantity which causes a rise of 30 mg. per 100 ml. in blood glucose, and is usually about 0.1 μ g. per kg. (13). Such an elevation of blood sugar usually returns to normal in 30 to 60 min., but the duration of the effect depends on the dose and route of administration. This effect of glucagon is not observed after hepatectomy (45). The immediate fall in liver glycogen has been confirmed (46, 47), and the distinction of this action from that of epinephrine by the fact that glucagon hyperglycemia is not blocked by dihydroergotamine (20) has been noted. The simultaneous loss of potassium during glycogenolysis induced by epinephrine and glucagon

has been described (48). In chronically treated animals, liver glycogen is normal (49) or elevated (50), and the effect on liver glycogen is altered in many conditions in which liver glycogen is reduced, as reviewed by Foa *et al.* (13). The identification of liver enzymes and their alterations under the glycogenolytic action of glucagon has been well advanced by Sutherland and his colleagues (26, 51 to 54). According to them, the liver inactivates phosphorylase by removing its phosphate (dephosphorylation). This inactivated enzyme can be reactivated by phosphokinase, and this reactivating process is accelerated or promoted by glucagon and epinephrine, thus resulting in increased glycogenolysis. Increased hepatic phosphorylase activity has been found after treatment with glucagon (46).

The action of glucagon on muscle and on the overall utilization of glucose remains uncertain as a result of conflicting results which are outlined by Foa *et al.* (13), Elrick *et al.* (33), and Tomizawa & Hyde (55). Glucagon protects dogs from alloxan diabetes, a protection which was abolished by the prior use of insulin and is presumably like the protective effect of glucose (56).

Glucagon appears to activate the adrenal cortex as shown by a fall in the circulating eosinophils (57) and of adrenal ascorbic acid (58); urinary corticoid excretion is increased (59). The possible importance of this induced or associated activity of the adrenal cortex is illustrated by the recent observation that the anabolic steroid, 17-ethyl-19-nortestosterone, abolished the hyperglycemic response to glucagon in man (60). An effect on gluconeogenesis from protein, direct or indirect, would add considerably to our thinking about the more sustained hyperglycemia of large doses and about a possible diabetogenic action (40). The adrenal cortex might be activated secondarily if glucagon prevented the destruction of corticotropin (61). Glucagon has been used to study the role of gastric hunger contractions and of the hunger and satiety mechanisms (62, 63), and it has been given intramuscularly or subcutaneously for the relief of insulin hypoglycemia in man (64, 65). The latter observations (64, 65) fail to compare its effectiveness to that of epinephrine which has long been used for this purpose.

THE BETA CELLS AND INSULIN

The subject of the islands of Langerhans and insulin has grown so that numerous subdivisions will appear in the following pages. Two broad questions confronting the physiologist are: "How do the islets, which produce and secrete insulin, work?" and "How does insulin work?" In connection with the first question some of the current findings about the islets and particularly the beta cells will be outlined.

The control of insulin secretion.—The important regulatory effect of glucose has been reviewed by Foa (66). Recently, the effect of glucose on insulin secretion has been measured in two ways. First, Whitney & Young (67) measured the insulinlike activity of plasma by the rat diaphragm method. They correlated the results obtained from glucose uptake (*a*) by diaphragms of normal rats from plasma of glucose-treated rats and (*b*) by diaphragms of glucose-treated rats from the usual buffer solution. Candela *et al.* found (68) the increased plasma insulin activity appeared after blood

sugar had returned to normal. Their results also suggested a change in the binding of insulin to tissue under the influence of glucose administration (67). Secondly, Pozza *et al.* (69) examined the response of dogs to a variety of sugars. They used both cross-circulation experiments and injection of the sugars into the pancreatic artery. The administration of D-glucose, D-galactose, or D-ribose was followed by a prompt hypoglycemia suggesting the secretion of insulin. D-Arabinose caused a delayed hypoglycemia, whereas D-fructose, D-mannose, D-xylose, L-arabinose, 3-methylglucose, D-glucosamine, galacturonic acid, and saline had no effect. They concluded that insulin secretion was stimulated by sugars which are utilizable and insulin-sensitive, and that there was no relation between chemical structure and the power to cause insulin secretion. Two reports in Russian (70, 71) on the possible role of the nervous system in insulin secretion have appeared.

Experimental diabetes.—Diabetes produced experimentally by repeated intraperitoneal injections of glucose has been reported by Link (72) and Yoshikawa (73). In Link's (72) experiment four pigs were probably made permanently diabetic with mild glycosuria and ketonuria, a description which might apply even to total pancreatectomy in this species. Islet damage was present but was difficult to evaluate for anatomical reasons.

Pancreatectomy in the rat, like alloxan diabetes, has provided a useful tool for investigators for more than 20 years. During this time efforts at complete extirpation of the rat's pancreas have been made but have not been satisfactory because of the death of the animals or the mildness of the diabetes. Thus, one recent effort (74) at complete pancreatectomy was followed by survival for periods up to 90 days and the capacity of the animals to gain weight. In contrast, Scow (75) has reported "total" pancreatectomy, i.e., leaving less than 0.5 per cent of the gland. His rats died within 48 hr. in diabetic coma, and hyperglycemia, glycosuria, ketonuria, severely fatty livers, and impaired absorption of food were observed. Food and insulin required for survival are described. Hypophysectomy in such rats (76) led to the expected modifications of the diabetes. From data in this article (76) the reviewer has calculated that in the depancreatized rat the average urinary glucose excretion during fasting was 1.6 gm. per kg. per day compared to none in the normal rat. The fasting nitrogen excretion of the untreated depancreatized rat was 0.61 gm. per kg. per day, not quite double the 0.36 gm. per kg. per day of the fasted normal rat. While the absolute amounts of glucose and nitrogen excreted vary in different species as Scow (75) notes, a striking increase in fasting nitrogen excretion has been the rule following pancreatectomy in all species studied by the reviewer, and this indicates the probable success of Scow's operation.

Two aspects of experimental diabetes have been investigated by Okamoto (77). First, he describes the production of experimental diabetes in rabbits by 87 compounds, mostly those containing zinc, and reports the effect of 86 substances tested for the prevention of alloxan diabetes. There was no simple relationship between structure and protective action. Second, he made male and female rabbits diabetic and bred them. Their young (F_1) were likewise made diabetic and were bred, and so on for subsequent generations. No

spontaneous diabetes occurred in F_1 , F_2 , and F_3 rabbits within 120 days after birth and before alloxan was given. However, all of 18 F_4 and all of 7 F_5 rabbits developed diabetes spontaneously. In most of them the diabetes was mild and intermittent; in three it was persistent, and these animals all showed degranulation, so-called hydropic degeneration, and atrophy of the beta cells in varying degrees. Okamoto recognizes the difficult problem of whether "a change effected by an external factor on somatic cells exercises any influence on germ cells or not." One hopes that he has not suffered the ill fate of Rowntree *et al.* (78) whose results with thymus extracts are now known to have been "artifacts".

The influence of strain, age, and sex on the diabetes produced by partial pancreatectomy in the rat (79) and an account of the histological changes in rats made diabetic with dehydroascorbic acid (80) have been described.

Effect of hormones on the islands of Langerhans.—Various types of hormonal treatment have been employed. The response of the islands as measured by changes in the insulin content (extractable insulin) of the pancreas, islet mass, and by histological changes will first be noted. In all such studies the question of whether the hormones exert their effects on the islets directly or indirectly must be kept in mind. Haist (81) found that hypophysectomy caused little change in the total amount of islet tissue in the rat's pancreas, although the pancreas as a whole is reduced in size. The daily administration of growth hormone increased the islet weight and the pancreatic weight in such rats; when it was given to intact rats, islet weight increased but the total pancreatic weight did not. When desiccated thyroid (82) was administered to intact or hypophysectomized rats, pancreatic weight and island weight increased. The fact that islet weight was increased by growth hormone (81) and by thyroid hormone (82) suggests that this response is an indirect one, i.e., something more than a hormone acting directly on the beta cells. Wrenshall *et al.* (83, 84, 85) have examined in detail certain technical factors influencing the extractable insulin of the pancreas. Unlike the dog and cat, the islands of the intact rat are quite resistant to the effects of growth hormone and are susceptible to the action of corticotropin (86). Degranulation and hypertrophy were the principal changes observed and were correlated with the occurrence of glycosuria at or near the time of autopsy. Similar results have been reported in partially depancreatized diabetic rats (87). Although growth hormone increased glycosuria at first, the glycosuria fell below the pretreatment amount despite continued treatment, and there was definite improvement in the microscopic appearance of the islands. When corticotropin was administered instead of growth hormone, there was increased evidence of cell damage. Chaikof & Campbell (88) have made a similar study in metahypophyseal-diabetic dogs. The administration of growth hormone, with insulin, increased the severity of the diabetes at first. Subsequently the requirements for insulin decreased from the pretreatment values. Histological examination during the period of reduced insulin requirement did not give evidence of regeneration of the cells of the islands of Langerhans. This difference from the behavior of the islands of the rat might be expected from past experience. Both groups of investigators have omitted

comment on the possible role of antihormones (89) in the physiological phenomena observed. Bennett (90) has also reported the effect of growth hormone on insulin secretion by the method of grafting the pancreatic vessels of a normal donor dog to the neck vessels of a normal or depancreatized recipient. The pancreas of the normal dog restored the blood sugar level of the depancreatized recipient to normal in about 3 hr. The amount of exogenous insulin needed to reproduce this effect provided an estimate of the rate of secretion. When the donor dog had been treated with growth hormone an increased insulin secretion could be demonstrated. Likewise, Altszuler *et al.* (91) found that under the influence of growth hormone more glucose disappeared from the plasma into the tissues, presumably a result of increased secretion of insulin, and Randle (92) reported an increased plasma insulin activity after growth hormone treatment. Lazarus & Volk (93) used partially depancreatized dogs with and without the supplementary administration of growth hormone or cortisone to study histological changes in the islets. Regardless of how diabetes was induced, early glycogen infiltration of duct epithelium in the pancreas and later glycogenization of the beta cells were found. They have also studied the effect of growth hormone, corticotropin, and hydrocortisone on the blood sugar and islet structure of the rabbit (94). The lesions, degranulation and glycogenization (hydropic degeneration), became more pronounced with increasing severity and duration of the diabetes. Buse *et al.* (95) produced diabetes and severe islet damage in the cat with fluorohydrocortisone. Bastenie (96), in his extensive review of steroid diabetes, illustrates marked hydropic change in a human island after two weeks of steroid diabetes. In their review article Ketterer, Randle & Young (97) include a good summary of the effects of growth hormone on the islands, and an editorial (98) affords a useful summary of the effects of cortisone on the acinar tissue.

Insulin and growth hormone.—Comprehensive reviews of the role of growth hormone in metabolism make it clear that growth hormone depends upon insulin for its full effect (97, 99, 100). At the same time, growth hormone is an insulin antagonist, so that "the anabolism of protein may be enhanced by insulin under conditions in which the action of insulin on carbohydrate metabolism is restrained" (97). Part of the evidence that growth hormone in some manner stimulates the secretion of insulin has already been cited (81, 90, 92, 99) and its diabetogenic effect under suitable circumstances is now an old story. The mechanism of this anti-insulin action has been extensively studied by de Bodo and his associates (91, 101 to 104), using the response of the blood sugar to a test dose of insulin. Measured in this way the insulin sensitivity of the hypophysectomized dog and the potent anti-insulin action of growth hormone in hypophysectomized dogs are clearly demonstrated. A similar abolition of insulin sensitivity in adrenalectomized-hypophysectomized dogs showed that the adrenal cortical steroids were not essential for this action of growth hormone (102, 103, 104). The investigators described the toxic effects in dogs of growth hormone from the pituitary glands of cattle, and the counteraction or control of these toxic manifestations by the use of corticotropin or cortisone. In view of the recent experiences on the

effectiveness of simian and human growth hormone in monkey (105) and man (106), respectively, it seems well to postpone discussion of the physiological meaning of these observations of de Bodo. Volk *et al.* (107) have used a modified glucose-insulin tolerance test to determine the insulin sensitivity of adrenalectomized or hypophysectomized dogs and the insulin insensitivity induced in normal dogs pretreated with growth hormone, corticotropin, or cortisone. More recently, de Bodo *et al.* (108 to 111) have undertaken a study of the influence of insulin and growth hormone on carbohydrate metabolism by the isotope dilution method (108). Normal (108) and hypophysectomized (109) dogs were studied as a foundation for observations on the action of insulin (110) and of the insulin sensitivity of the hypophysectomized dog (111). Here, only the last report on the relation of the anterior pituitary and insulin will be noted. In the hypophysectomized dog the same dose of insulin caused a greater removal of glucose from the plasma than in the normal dog. At the same time the hypoglycemic state failed to bring about the release of glucose from the liver in the normal amount. In short, two factors are acting together to produce insulin-sensitivity, viz., an increased uptake of glucose by the tissues and a diminished outflow of glucose from the liver. Treatment of the hypophysectomized dogs with growth hormone, as noted above (91), increased the rate at which glucose was released from the liver and the uptake of glucose by the tissues. In like manner, treatment of hypophysectomized dogs with cortisone or hydrocortisone increased the rate of outflow of glucose from the liver and the glucose uptake by the tissues [Altszuler *et al.* (112)]. The authors' conclusion was that the primary effect was on the liver, at least under steroid treatment (113), and that the increased uptake of glucose by the tissues was the secondary effect of an increased endogenous secretion of insulin.

Houssay & Penhos (114) demonstrated that growth hormone and prolactin produced a rise in blood sugar from normal to 200 mg. per 100 ml. or more in adrenalectomized-hypophysectomized dogs. Growth hormone was more active than prolactin, and in general these results are in harmony with those of Sinkoff *et al.* (102). Campbell *et al.* (115) examined many effects of growth hormone in dogs. The marked diabetogenic action was confirmed and in diabetic animals the extractable insulin of the pancreas was 1 to 2 per cent of the average normal value. Incidentally, they (115) and Warner *et al.* (116) found changes in the plasma proteins resulting from treatment with growth hormone. Growth hormone and cortisone in combination caused marked hyperglycemia in alligators (117).

Insulin and adrenal cortical hormones.—Some of the effects and relationships of adrenal steroids and insulin have just been cited (102, 103, 104, 112, 113). Thorn, Renold & Winegrad (118) have summarized many observations bearing on the interaction of adrenal steroids and insulin, and Froesch (119) has reported the response of blood corticoids in man to insulin hypoglycemia. Ingle *et al.* (120) have studied the diabetogenic effect of certain progesterone derivatives in the rat, and Penhos & Cardeza (121) produced temporary diabetes in partially depancreatized dogs and rats with prednisone or prednisolone. In animals of both species which became resistant to the steroids there

was hyperplasia of the islands of Langerhans. Hausberger & Hausberger (122) measured the overall utilization of glucose before and during steroid diabetes in guinea pigs. Glucose utilization was calculated by the conventional formulae based on carbohydrate intake, urinary glucose, and urinary nitrogen excretion. The animals made diabetic with steroids "compensated for the glucose loss fully by increased food intake and increased gluconeogenesis" so that actual glucose utilization was not diminished. Although there were varying degrees of islet damage, they noted the great regenerative capacity of the islands in this species, a fact which presumably explains the failure to produce permanent diabetes in the guinea pig. A reverse situation, namely the modification of alloxan diabetes in rats by suppressing adrenal cortical function with 2,2 bis-(para-chlorophenyl)-1,1-dichloroethane (DDD) has been described (123). McArthur *et al.* (124, 125) studied the response of the adrenal cortex to pancreatic diabetes by the use of modern methods of recording adrenal function. The findings in depancreatized dogs were related to those in diabetic dogs treated with corticotropin and in adrenalectomized-depancreatized dogs.

Other relations of the islands.—Bloom & Russell (126) have made a detailed comparison of the effects of epinephrine and norepinephrine on carbohydrate metabolism. Their report contains a description of the adrenal medullary response to hypoglycemia induced by insulin, and the lack of this response in demedullated animals. Engel *et al.* (127) described marked impairment of glucose tolerance and resistance to the hypoglycemic action of insulin in rats made diabetic by sodium fluoroacetate. The effects of alloxan in animals with atrophic acinar tissue following ligation of the pancreatic duct have been studied and reviewed (128). Maske *et al.* (129, 130) have described the distribution of zinc in the islands of Langerhans. More zinc is in the islets when insulin is being stored. By using the relatively pure islet tissue of fish, they have shown that most of the insulin is in the mitochondrial fraction, which contained enough zinc to precipitate that amount of insulin. The concentration of zinc in the main microsomal fraction is even higher.

Landau & Renold (131) measured the concentration of radioactivity in the islets after the administration of alloxan 2-C¹⁴. The essence of their results was that the concentration was no greater in the pancreas (or islets) than in other tissues. The conclusion was that a greater susceptibility of the beta cells, rather than an unusual accumulation of the drug, was the factor underlying the toxic action of this drug. Another method of entering the beta cell, so to speak, has been the demonstration of insulin in the islets of some species by means of the fluorescent antibody technique (132), a procedure that ought to be useful in the future.

In man, little is known of the changes which occur in the islands of Langerhans in relation to the development, exacerbation, or improvement of diabetes mellitus. Lukens & Dyer (133, 134) obtained biopsies on three diabetic patients: (a) one who recovered following the removal of a pheochromocytoma; (b) one who did not recover following the removal of the same type of tumor; (c) a patient with Cushing's syndrome at the time of adrenalectomy. The variable lesions encountered in this limited experience

indicate that many more observations are needed to give one an idea of how the islands may behave in man. The problem of estimating the severity of diabetes, experimental and clinical, has been discussed (135, 136). Using autopsy material, Hartroft, Wrenshall & Bogoch (137, 138) have reported the weight and extractable insulin of the pancreas in nondiabetic and diabetic patients. In the diabetics there was a nearly direct proportionality between the frequency and intensity of beta cell granulation and the amount of insulin extracted per unit weight of pancreas. Less insulin per unit area of islets as well as diminished islet area was demonstrated in the diabetics. Finally, two reports have appeared (139, 140) on the survival of patients for ten years or more after total pancreatectomy. One of these patients (139) has developed diabetic retinopathy. It is possible that modern surgery may cast new light on the relation between the metabolic disorders and the vascular complications of diabetes mellitus. Morphological changes in the blood vessels of the cheek pouches of hamsters after three months or more of alloxan diabetes (141) may advance the experimental attack on this problem. Two cases of the unusual combination of islet cell tumor and diabetes have been reported (142), and the changes in the islands of Langerhans in patients with occlusion of the pancreatic duct have been examined (143).

Tolbutamide and the islands.—The whole subject of the hypoglycemic (meaning hypoglycemicogenic) sulfonylureas is beyond the scope of this chapter. Four recent symposia (144 to 147) are noted. For the physiologist interested in the islands of Langerhans, these substances, of which tolbutamide has been most investigated in the United States, provide the first pharmacologic influence on islet (beta cell) function other than the damaging action of alloxan and such toxic substances.

The evidence for a pancreatic site of action of the sulfonylureas has been summarized by Levine & Sobel (148). (a) The drugs are ineffective in depancreatized animals (148 to 151). This dependence on the secretion or presence of insulin has been emphasized by the observation that adrenalectomy leads to a greatly increased sensitivity to the blood sugar lowering action of the sulfonylureas (149, 150, 152), yet even this increased susceptibility is abolished when the adrenalectomized animal is depancreatized (152). In contrast to the adrenalectomized animals, hypophysectomized animals, which are more sensitive to insulin, respond to the sulfonylureas to about the same degree as normal animals (149, 150, 151). The absence of hypoglycemia after administration of these drugs in Houssay animals (149, 150, 151) as well as in adrenalectomized-depancreatized animals (152) emphasizes the fact that the sulfonylureas do not replace insulin, to which such doubly-operated animals are extremely sensitive. (b) Cross-circulation experiments have also indicated the presence of insulinlike activity in the pancreatic vein blood of donor animals treated with sulfonylureas (153). (c) Hepatectomized dogs, maintained on intravenous glucose, have a hypoglycemic response after tolbutamide which resembles that seen in normal dogs. This response is logically attributed to the release of insulin (148, 154). (d) The hypoglycemic effect of small doses injected directly into a pancreatic artery (155) adds further evidence for an effect of the sulfonylureas on the islands. The doses ad-

ministered in these experiments were too small to produce any effect if administered intravenously or orally. (e) Histological changes have been described in the beta cells of the islets (156, 175). Degranulation of the beta cells was induced by tolbutamide but was not seen after insulin hypoglycemia (156), indicating that the change in the cells was a primary effect of the drug and not secondary to the fall in the blood sugar level. A similar degranulation has long been known to occur after the administration of glucose. In spite of the difficulties in relating structural changes to function, this combination of events is further evidence for the release of insulin in response to tolbutamide. In addition, experimental diabetes varying in degree and duration has been studied (157). In dogs and rabbits whose beta cells were completely degranulated by preceding diabetes, no hypoglycemia occurred after tolbutamide. (f) This relationship between the degree of insulin deficiency, histological appearance, and responsiveness to tolbutamide is paralleled in clinical experience. The most insulin-dependent diabetic patients, with little or no extractable pancreatic insulin, do not respond to the sulfonylureas. As a result, it has been suggested that the response to tolbutamide be used as an index of the degree of insulin deficiency. Unger & Madison (158) found that even mild diabetics, with nearly normal levels of fasting blood sugar, responded to tolbutamide more slowly than normal subjects. (g) Finally, an increased insulinlike effect of the plasma of animals treated with carbutamide has been claimed (159, 160) and denied (161). Plasma insulin was measured by the action of plasma on the normal rat diaphragm, and by the fact that the diaphragms of rats pretreated with carbutamide had a significant increase in glucose uptake (162). This effect was not seen when carbutamide was added to the normal rat diaphragm *in vitro* (159). The failure to find an increased plasma insulin effect (161) is in agreement with other studies indicating a lack of peripheral action of the sulfonylureas. However, the bulk of the evidence indicates that there is increased insulin secretion but that the amount, measurement, and significance of this require further study.

In addition to bringing about the release of insulin by the islets, the sulfonylureas act upon the liver as reviewed by others (144 to 148). This chapter will note only the fact that the presence of insulin is essential for this response. This is indicated by the lack of hypoglycemia when depancreatized animals are treated with sulfonylureas, as already cited. Houssay & Migliorini (163) and Caren & Corbo (164) have found that carbutamide (163) and tolbutamide (164) increased the intensity and duration of action of administered insulin in totally depancreatized dogs. A feature of these experiments was the small dosage of insulin used, viz. 0.04 (163) and a range of 0.01 to 0.25 (164) units per kg. per hr., and the fact that a large dose of insulin obscured this effect of tolbutamide (164). Other effects of sulfonylureas on the liver, summarized elsewhere (148), such as changes in certain liver enzymes, have not been found to occur in depancreatized animals. They probably represent an influence of the drugs on what might be called a liver cell-plus-insulin system.

In spite of some discrepancies, there is general agreement that the sulfonyl-

ureas do not act on muscle nor do they potentiate the peripheral action of insulin. Data on this topic are contained in the symposia (144 to 147). Pre-treatment of rats with carbutamide did not alter the response of the diaphragms of treated animals to insulin (165), and tolbutamide did not alter the rate of disappearance of labelled glucose in dogs (166). Studies in man strongly support the concept that these drugs are without peripheral action. Craig & Miller (167) found no alteration in the rate of utilization of labelled glucose, and Segal *et al.* (168) observed no change in the rate of disappearance of those pentoses which respond to insulin when human subjects were treated with tolbutamide. The matter of a peripheral action of the sulfonyl-ureas has been reviewed by Stadie (169).

INSULIN

The chemistry of insulin.—The outstanding work of Sanger and his colleagues (170, 171) and of other students of this subject has been reviewed (172, 173, 174). No effort has been made to survey such fundamental chemistry in this article, but some of the applications of our knowledge of the chemistry of insulin may be seen in the studies on the immunological behavior of insulin which will be noted later.

The action of insulin.—This is a topic of the greatest interest among students of diabetes, insulin, and metabolism; and many excellent reviews have appeared (175 to 186). The recent dates, broad bibliographies, and competent organization of these articles make repetition unnecessary. In general they assemble the recent positive findings as meaningfully as possible. On the chance that a different arrangement may serve some purpose, some of the current facts will be placed against the background of insulin deficiency. This merely means that in a depancreatized animal or severely diabetic patient it is not yet possible to say what is the primary or most specific defect resulting from the absolute or relative deficiency of insulin. In particular, one cannot answer such questions as, "In what tissue(s) and for what reaction(s) is insulin essential?"

Brain.—During the survival of the untreated depancreatized dog, the central nervous system functions normally to outward appearance until the secondary effects of diabetic acidosis develop. When survival after pancreatectomy is prolonged by the use of other species or by the combination of hypophysectomy with pancreatectomy, the brain continues to function until death occurs from cachexia. In the treated diabetic patient, the brain appears to remain normal aside from the possible development of vascular complications. Why the brain should escape the hazards of the disease and how the peripheral neuropathy develops are not yet understood. Such general comments are in harmony with early observations that the R.Q. of the brain remains at 1.0 in the diabetic. Recent reports continue to support the concept that the brain is independent of insulin. This is reviewed by McIlwain (187), Stadie (177), and by Geiger & Yamasaki (188) who found no binding of insulin by the brain. Elgee *et al.* (189) also reported the absence of appreciable amounts of labelled insulin in the brain. Park *et al.* (180, 190) have examined the effect of insulin on the transport of several hexoses and

pentoses into various tissues and found no effect of insulin in brain. Such results, added to many past studies, lead to the conclusion that there are apparently no metabolic pathways in the brain which are dependent on insulin, and this seems to be true when long periods of time are taken into account.

Heart.—The comprehensive studies of Russell & Bloom (191) on the hormonal control of cardiac glycogen led them to conclude that the level of glycogen was independent of insulin and was significantly under the influence of growth hormone. Studies in Houssay animals (192) add further evidence for the independence of cardiac glycogen and insulin. The observed correlation between the level of blood glucose and that of cardiac glycogen (192) is compatible with the demonstration that the myocardium is, at least to some extent, independent of insulin for glucose transport. Thus, Fisher & Lindsay (193) found that the rate of penetration of galactose into the perfused heart bore some relation to its external concentration at the same time that they noted an increased intracellular penetration of galactose and glucose under the influence of insulin. In Park's (180) experiments insulin had a marked effect on the distribution of insulin responsive sugars in the hearts of eviscerated rats. When glucose was used the concentration of free glucose in the muscle was increased. Thus, the heart responds to insulin but may still be less dependent on the hormone. Sacks & Bakshy (194) also noted some penetration of sugars into the heart after insulin treatment. At any rate, after pancreatectomy the heart seems to carry on well for days or weeks. In diabetes mellitus it is not the myocardium but the coronary arteries which suffer from an unknown long-term effect of diabetes.

Muscle.—The effects of insulnia, or the lack of it, on muscle have been studied in numerous ways. The increased uptake of glucose by muscle has been well demonstrated and reviewed by Levine & Goldstein (175). The effect of insulin in promoting the transport of glucose into muscle has become a central concept of its function as shown by other reviews (176, 181, 183). Ross (183), whose studies of the effect of insulin on the transport of glucose from the blood to the aqueous humor of the eye have been a major contribution to the transport or "permeability" hypothesis, has concluded: "Present evidence suggests that insulin accelerates the transport of glucose across the cell membrane and that this transport represents a principal locus of insulin action." Only a few recent observations concerning this relation of insulin to muscle and extrahepatic tissues will be noted. Gourley (195) has confirmed in the frog the combination of insulin with muscle and has suggested that there may be two types of reactive site, one concerned with oxygen consumption at which insulin unites rapidly and another concerned with potassium transfer at which insulin combines more slowly. Fritz *et al.* (196) studied the effects of insulin and epinephrine on glucose disappearance in eviscerated dogs. In insulin-treated animals epinephrine inhibited the disappearance of glucose at normal and hypoglycemic levels of blood sugar, but in non-insulinized animals it had no effect on glucose disappearance. The action of insulin on the distribution of galactose was not altered by epinephrine. Additional studies on the disposal or transport of glucose from the blood have been based on the use of isotopic glucose or of pentoses, and these methods

have permitted observations in man. Dunn *et al.* (197) review the previous use of a tracer dose of uniformly labelled glucose-C¹⁴ for studying the effect of insulin. They adapted this procedure to measure the rate of entry and the rate of removal of blood glucose. Two applications of this method to the study of insulin have already been cited (108, 110, 166). All agree that insulin causes an increased rate of removal of glucose from the blood, and transport into tissues was also shown by the incorporation of labelled glucose into muscle glycogen and into fat (166). In addition to substantiating the earlier work on the eviscerated animal (175) and the eye (183), the simultaneous effect of insulin on the entry of glucose into the blood was estimated. Here there is disagreement. (a) Some (110, 197) conclude that insulin causes an immediate inhibition of the entry of glucose into the blood for 10 to 20 min. (b) Ashmore and co-workers (166) find only that insulin hypoglycemia is associated with an increased production of glucose by the liver, i.e., an increased entry of glucose into the blood, an effect which all observe during the main period of insulin action. (c) During the whole period of insulin action there is an increased rate of removal from the blood (110, 166, 197, 198). The part played by the hepatic and peripheral sites of insulin action have been calculated. "From one-fourth to one-half of the total drop in blood sugar was estimated to be due to inhibition of entry, and the remainder to increased removal" (197). Dunn *et al.* (197) proposed a dual action of insulin at the cell surface, dual in the sense that transport was influenced in different ways in the liver and periphery. Henderson *et al.* (198) have described a smaller and slower effect on the hepatic release of glucose. Such measurements of the hepatic action of insulin agree with earlier work based on hepatic vein catheterization (199, 200), but the determination of the magnitude of the response and the ultimate meaning of these new methods used to measure the hepatic effect will require further investigation.

Sacks & Bakshy (194) studied the effect of insulin on the distribution of pentoses in the tissues of nephrectomized cats. L-arabinose and D-xylose had an extracellular distribution in muscle, and partial intracellular penetration occurred after the administration of exogenous insulin. The results with pentoses led them to conclude that if glucose were transported in a like manner such transport preceded phosphorylation. Using pentoses in man, Segal *et al.* (201) confirmed the fact that insulin affected the volumes of distribution of D-xylose, L-arabinose, and D-lyxose. The fact that D-lyxose was slightly responsive to insulin suggested that the present concept of the relation between the structure of sugars and their reactivity to insulin may need revision. Beloff-Chain *et al.* (202) report that no differences were observed in the metabolism of glucose by diaphragms from normal and diabetic rats, findings which may be explained by the mildness of diabetes in their animals as shown by slight inconsistent loss of weight and by their contrast to the severe diabetes described by Scow (75). In alloxan diabetic acidosis a decrease in the potassium and water content of skeletal muscle has been found (203), and the rat diaphragm contains both insulin-responsive and insulin-nonresponsive glycolytic systems (204). Insulin increases the rate of glucose uptake by uterine smooth muscle, an effect which was altered

when the uterus was conditioned by pretreatment with sex hormones (205).

Undoubtedly insulin exerts important effects on muscle, which will be further described in connection with protein metabolism. Depancreatized animals, Houssay animals, and diabetic patients all suffer somewhat from muscular weakness. Yet the muscles continue to function remarkably well in the absence of insulin and no myopathy results from diabetes. This leads to the questions: "How essential is the action of insulin for the survival of muscle? Could insulin deficiency in muscle lead to death of the animal?" Important as the effects of insulin in muscle are, one may borrow the language of the bacteriologist and ask: "Is the relation of insulin to muscle obligatory or facultative?"

Exercise.—Pursuing earlier studies, Ingle *et al.* (206) found that stimulation of muscle exerted an insulinlike effect on the tolerance to glucose of the eviscerated rat, and that a combination of muscle work with insulin in suitable dosage was greater in its effect on glucose metabolism than either exercise or insulin alone. In the eviscerated rat, insulin suppressed the rise in plasma amino acids but muscle work did not do so. This result, added to other observations, indicates that muscle work and insulin produce their effects on blood sugar by different means. Levine and his colleagues (175, 207) have examined the effect of exercise on the distribution of sugars. (a) Those sugars responsive to insulin were responsive to exercise. (b) This result of exercise occurred in eviscerated rats and dogs even in the absence of the pancreas. (c) Finally, they demonstrated that this effect on the distribution of sugars might be humoral, since the stimulation of one group of denervated muscles still led to the distribution of galactose in a volume corresponding to that of total body water. Their conclusion that "this action of muscular work is independent of insulin" is valid for their experiments but will be accepted with reservation by those who recall the early work of Marble (208) and Richardson (209), who agreed that exercise raised the blood glucose values in severely diabetic patients. In hindsight, Richardson's (209) observation that a dose of insulin too small to affect the blood sugar was made effective by exercise favors the idea that exercise is insulin-enhancing rather than insulin-independent. Few would deny that the relation between insulin and exercise remains an intriguing problem.

The liver.—Some of the work concerning the effects of insulin on the liver has already been cited (108 to 113, 164, 166, 197, 199, 200) and the relation of the liver to the action of insulin is outlined in a number of the reviews (175, 176, 177, 181, 182, 183). As far as the output of glucose by the liver is concerned, insulin causes an initial suppression according to some workers and a later increase in the entry of glucose into the blood according to all reports. The extent to which a change in glucose output by the liver is related to insulin and to the level of blood glucose itself remains uncertain. The promptness of the response (110, 197) suggests that it may be the result of the hormone itself, and the high concentration of administered insulin in the liver (189) points to its importance for this organ. In depancreatized-nephrectomized dogs, and in nephrectomized control animals, Issekutz (210) has studied the relation of the blood glucose level to the uptake and release

of glucose by the liver. During hyperglycemia a high glucose uptake by the liver was calculated in the controls, but in diabetic dogs the liver seemed to release glucose even at a very high blood sugar level and no comparable uptake of glucose was observed. Madison & Unger (211) have re-examined the response to the administration of insulin via the portal or systemic circulation. When insulin was injected into the portal vein it caused as much arterial hypoglycemia as the same dose given via the peripheral circulation, yet the utilization of glucose as indicated by the arteriovenous difference was much less than when the peripheral route of administration was used. In short, there appear to be a hepatic and a peripheral action of insulin, and the magnitude or balance of each component depends in part on the route of administration. Haft & Miller (212), using isolated perfused rat liver, recorded the changes in alloxan diabetes as: decreased lipogenesis from acetate, increased urea formation, and increased incorporation of acetate into carbohydrate. In animals which were not too ketotic, insulin partially corrected the depressed lipogenesis and increased the removal of glucose from the perfusate.

Using rat liver slices Renold *et al.* (213) have contributed greatly to our understanding of the role of time in the action of insulin. Like others, they noted the prompt action of insulin on the rat diaphragm *in vitro*. In contrast to the diaphragm, they observed no effects of insulin added to liver slices *in vitro*; but between 6 and 24 hr. after the administration of insulin to diabetic rats, the metabolism of their liver slices was restored although full return to normal required about 24 hr. They introduce the concept that there is an immediate effect of insulin at the periphery and a slower effect on the liver, which they consider may be the metabolic adaptation of the liver to the demands created by the changes in the periphery. These investigators have also examined the pathways of glucose-6-phosphate metabolism in liver slices from normal and diabetic rats (214). In diabetic animals only one-quarter as much glucose was phosphorylated as by normal rat liver. The changes in the individual pathways by which the glucose-6-phosphate was metabolized were quantitatively determined. In summarizing their results and those of others, Renold *et al.* note that in insulin deficiency there is: decreased phosphorylation of glucose; an increase in glucose-6-phosphatase activity; a decreased formation of glycogen from various substrates; a reduction in glucose-6 phosphate dehydrogenase, and in metabolism by way of phosphogluconate oxidation. More recently the same group (215) has studied the distribution of radioactive glucose in plasma water and liver water. Labelled glucose was distributed in total liver water whether the liver was taking glucose from or delivering it to the plasma. Many other sugars entered the liver. Alloxan diabetic rats also had free equilibration of glucose between liver and plasma water. This permeability led them to conclude that, in the liver, insulin alters intracellular enzyme functions rather than the permeability of the cell wall, which it alters in muscle.

More information on fat synthesis by liver slices of normal and alloxan diabetic rats has appeared. Felts *et al.* (216) compared the conversion of glucose-1- C^{14} and glucose-6- C^{14} to CO_2 and fatty acids in the livers of normal,

diabetic, and insulin-treated diabetic rats. Insulin treatment several hours before diabetic animals were sacrificed [cf. (213)] augmented the recovery of $C^{14}O_2$ from glucose-1- C^{14} much more than it did from glucose-6- C^{14} . While both types of labelled glucose were incorporated into fatty acids under insulin, the effect was more pronounced with C-6 than with C-1 labelled glucose. The use of these selectively labelled forms of glucose showed that there was a threefold increase in the proportion of labelled fatty acid carbon that arose via the phosphogluconate pathway. By the injection of C^{14} -labelled tripalmitin, Lossow *et al.* (217) showed that liver slices of insulin-treated diabetic rats converted less labelled palmitic acid to carbon dioxide and acetoacetate than did the slices of untreated rats. They also found that insulin failed to reduce the oxidation of labelled octanoate, and they propose the concepts that insulin spares palmitic acid mainly by restricting its breakdown but also by promoting its synthesis, and that both fat sparing and lipogenesis are the result of the increased utilization of glucose by the liver. Finally, Bauman *et al.* (218) have studied hepatic lipogenesis in diabetic-hypophysectomized rats. The impaired fat synthesis of diabetes was partially restored by hypophysectomy. The results add information on the part played by substrate, diet, nutritional condition, and species in animals with partial insulin deficiency. Even after hypophysectomy, insulin is important in lipogenesis, as seen in many other types of experiment. Thus the gain in weight of insulin-treated hypophysectomized rats was almost wholly accounted for by fat deposition (219), and insulin increased aspartic-glutamic transaminase in the liver. In connection with both liver and muscle, the chemistry and metabolism of glycogen have been reviewed (220).

The fact that so many reactions in the liver are influenced by insulin suggests that this organ might well be a place where the action of insulin is essential. The metabolism of the storage forms of food, i.e., lipogenesis, ketogenesis, and the formation and breakdown of glycogen are processes which occur in starvation and refeeding. Many of these transformations include some degree or type of glucose oxidation, and the oxidation of glucose is necessary for the maintenance of tissue protein. If the liver is independent of insulin for the intracellular transport of glucose (215), it might be all the more dependent on the hormone for some essential intracellular reaction(s).

Adipose tissue.—The depletion of fat stored in adipose tissue is a striking feature of depancreatized animals, and the rate at which fat is mobilized in the absence of insulin varies with species and other conditions. One result of diabetes is an elevation of blood lipides which has been found to occur in the rat (221) as in other species. Accelerated lipogenesis in adipose tissue and liver has been studied in mice given labelled glucose after a dose of insulin (222). Large doses of insulin enhanced peripheral lipogenesis whereas the liver responded more than the periphery to small doses. Progress continues in the study of adipose tissue *in vitro* (182). Using mesenteric tissue, Itzhaki & Wertheimer (223) found that insulin did not affect the oxygen consumption of adipose tissue although this was increased by fasting or refeeding after a fast. They emphasize the importance of the nutritional state in such studies. The use of the epididymal fat pad of the rat (224, 225) may prove

to be of considerable value. This tissue seems to be peculiarly well suited for *in vitro* work. Like the rat diaphragm, it responds promptly to insulin added to the medium and is even more sensitive. The fat pad may thus serve as an improved bioassay of insulin and as a means of following the metabolic fate of various substrates in adipose tissue. Like others (223), Renold *et al.* (224) found that insulin increased glucose utilization and fatty acid synthesis in adipose tissue. In diabetes there appears to be no hazard primarily related to the mobilization of fat, except for the increased rate of mobilization which resembles the response to starvation. (The secondary dangers of severe ketogenesis are here disregarded.) On the other hand, an abnormality of adipose tissue which prevented the conversion of glucose to fatty acids could be a serious factor in diabetes. This has been postulated in the "lipo-atrophic diabetes" described by Lawrence (226) and others (224), but the simultaneous liver disease of these patients forbids final conclusions about the function of their adipose tissue. In the usual diabetic patient adipose tissue responds well to insulin, so that the deficiency of the hormone rather than of the end-organ is assumed.

Insulin and protein metabolism.—The effect of insulin on tissues other than those mentioned above is occasionally reported but will not be reviewed here. Instead it seems more pertinent to note the relation of insulin and protein metabolism, as protein is common to all living tissues. The essential part played by insulin in protein metabolism has been reviewed (227, 228, 229) and is emphasized by recent investigations. These have included the use of labelled amino acids to ascertain the influence of insulin and glucose on protein metabolism. Munro (228) has pointed out the relation of carbohydrate and insulin to the utilization of dietary protein and in the sparing of endogenous protein. He demonstrated that muscle was a site of the protein sparing action of carbohydrate; his failure to observe hepatic protein synthesis probably resulted from the experimental conditions. Krah1 (181, 227) and Sinex *et al.* (230) have shown this effect of insulin on the uptake of amino acids by muscle *in vitro* and also found that this occurred without the addition of glucose to the medium. As Krah1 (181) states, this shows that insulin probably has some action on protein anabolism apart from the transport of glucose. Turning to the liver, Prudden *et al.* (231), using perfusion of the isolated organ, found that insulin stimulated the uptake of amino acids and that growth hormone had an even greater effect. The hepatic and plasma proteins, carbon dioxide and urea production, and glycogen and lipide formation were measured. Although they noted that their results did not localize the chemical site of action of the hormones, they clearly demonstrated an action on protein anabolism in the liver. By examining the effects of insulin in Houssay cats, Lukens (192, 232) found that in some instances nitrogen retention occurred in the absence of growth hormone. Such nitrogen retention was not related to the calculated glucose utilization during insulin administration but seemed to be related to the greater increment in glucose utilization in the more "depleted" animals. In insulin-treated animals a striking increase in hepatic protein was demonstrated by the increase in liver weight and nitrogen content above the levels found in untreated Houssay

animals. As an organ most actively concerned in the synthesis of protein, the liver is obviously influenced by the anabolic hormones (192, 231). Krah1 (227) has extensively studied the peptide synthesis of normal and diabetic rat liver slices and the anabolic action of insulin in this preparation. Salter, Best, and Lawrence (233, 234) have recorded the anabolic action of insulin in hypophysectomized rats in terms of the effects on epiphyseal cartilage, body protein, and nitrogen retention, and they found that the liver weight of hypophysectomized controls was doubled by insulin treatment (233). They noted that the effect of the increased caloric intake caused by insulin treatment had not been fully controlled. This has been done subsequently by Wagner & Scow (235) by giving insulin to force-fed hypophysectomized rats and by increasing the food intake without exogenous insulin in control rats. Insulin was ineffective, but increased food intake led to a gain in body protein of 2.7 gm., confirming the earlier figure of 2.6 gm. (233). The relation of insulin in varying dosage to the nitrogen-retaining action of growth hormone in 95 per cent depancreatized rats (236) confirms the fact that for the optimal effect of growth hormone adequate insulin must be present. Species differences in the response of rats to these hormones are contained in this report. The possible relations between insulin, growth hormone, and other anabolic agents are discussed elsewhere (227, 232, 233).

Insulin promotes all of the important ways by which carbohydrate is utilized. The reactions of glucose concerned with its direct oxidation, glycogen formation, and fat synthesis are known. The pathway by which glucose and insulin promote protein synthesis remains unknown. Perhaps if this were known, the action of insulin could be more completely studied, obligatory and optional effects of the hormone might be distinguished, and the importance of this action of insulin in all tissues and in particular tissues might be evaluated.

Metabolism of insulin.—This heading applies broadly to a variety of studies on the distribution, duration in the body, state or form of insulin *in vivo*, excretion, and inactivation, which is discussed principally in the study of insulinase. The distribution of insulin in various tissues has been reported (189) and its degradation examined by the use of labelled insulin. The degradation of insulin is rapid and appears to occur in large part by the action of a proteinase, called insulinase for convenience, since its specificity for insulin is still disputed. The work on insulinase has been reviewed by Mirsky (237). In general, the degradation of insulin in tissues has been studied by conventional bioassay methods after insulin and tissue preparations have been incubated, and by the release of I^{131} -labelled fragments of labelled insulin into the nonprotein fraction of an extract (182, 237, 238, 239). The most active site of degradation of insulin appears to be the liver (237, 240, 241) and this has led to efforts to identify the fraction of the liver which is responsible. Liver extracts are effective (242, 243) and the intracellular, heat-labile nature of the inactivating agent has been described (244). The rate of degradation of insulin in the rat was increased by the administration of thyroxine and greatly diminished after thyroidectomy (245) or hypophysectomy (246), whereas adrenalectomy or the administration of growth

hormone caused little alteration (246). Mirsky (237) first described insulinase-inhibitor in liver extracts, and the inhibition of the degradation of insulin in the liver by this means was accompanied by increased physiological activity of insulin. Since then, competitive (hepatic) and noncompetitive (chemical) inhibition of insulinase activity has been described (237, 247). Mirsky (237) classifies tolbutamide as a weak noncompetitive inhibitor of insulinase, an effect which is probably only a small part of its hypoglycemic action. Long lists of these inhibitory substances are contained in his review (237). The distribution of insulin- I^{131} has been studied in eviscerated-nephrectomized animals (248) in which the degradation of insulin is relatively slow because of the removal of the liver and kidney which are the most active organs in its degradation. Insulin then was distributed in the extracellular compartment, and its activity on the blood sugar paralleled the concentration in the plasma. Wick & Drury (249) have also examined the retarding effect of native insulin on the decay of labelled insulin. The volume of distribution of iodoinsulin is reduced by the addition of unlabelled insulin. They propose a "sequestering of part of the tracer compound by a process that can be saturated by added native insulin." In any case, this contributes to the method of using iodoinsulin for distribution studies.

Insulin in blood.—This heading is used to include several aspects of insulin, insulin-protein complexes, and insulin antagonists which may be hormonal or immunologic, i.e., antibodies against insulin. Vallance-Owen (250) and Randle (251) have reviewed the various methods of assay. These assays have usually been performed with plasma, but serum and whole blood have been used in some instances. The methods of assay have been: (a) the alloxan-diabetic, hypophysectomized-adrenalectomized rat (ADHA rat) which is difficult to prepare and somewhat unreliable; (b) the rat diaphragm method has been most extensively employed; and (c) the epididymal fat pad of the rat has just been applied to insulin assay (224, 225). Methods and results have been reviewed (250 to 253). When untreated plasma is used the plasma insulin activity, or effective plasma insulin, is measured, since bioassay records the net result of insulin and insulin antagonists. In general, the concentration of insulin in blood increases after a carbohydrate meal, after injection of insulin, in hypoglycemia caused by islet cell tumors, and in acromegaly (97, 250 to 253). The sensitivity of the *in vitro* assays ranges from 10 to 200 microunits per ml. of plasma. It was found that the action of insulin added *in vitro* to the plasma of uncontrolled diabetics, especially those in acidosis, was inhibited. This was true when there had been no previous treatment with insulin and hence no opportunity for antibody production. The nature of this physiological antagonism was studied by Vallance-Owen (254) who found no detectable insulin in the plasma of depancreatized cats and marked inhibition of insulin added *in vitro* to such plasma. This inhibition was completely removed when pancreatectomy was combined with either hypophysectomy or adrenalectomy, indicating its endocrine origin. Experiments on the administration of growth hormone or cortisone to the doubly operated cats led to the conclusion that both growth hormone and adrenal cortical steroids were needed to produce this antagonistic action of plasma.

Whitney & Young (255) observed an increased glucose uptake when normal rat diaphragm was incubated with serum from hypophysectomized rats, the uptake reverting to normal after treatment with growth hormone or cortisone. Serum from normal rats pretreated for two weeks with both growth hormone and cortisone depressed the uptake of glucose by normal rat diaphragm. Field, Stetten, and Tietze (256, 257) have advanced the study of insulin antagonists in diabetic acidosis in man. Insulin activity was abolished by alpha-globulin fractions of the serum and slightly depressed by beta-globulins [cf. (254)], and the antagonist was not a lipoprotein (254, 256). After the diabetic acidosis had been treated the insulin antagonist was no longer present in the serum. They also found that this antagonist did not prevent the binding of insulin by diaphragm and did not exert insulinase activity (256). It was inactivated by chymotrypsin but not by trypsin, and on starch-block electrophoresis migrated with the alpha-globulin fraction of the serum proteins (257). It has no glucagonlike activity and could inhibit human insulin as well as beef-pork insulin in the diaphragm assay. One technical problem has been the influence of dilution on the diaphragm assay. The method has been modified and this problem examined by Willebrands *et al.* (258) who recommend a standard dilution of ten times.

Antibodies to insulin have been studied by Moloney & Coval (259), who showed that the intravenous injection of pig, ox, or sheep insulin caused anaphylactic shock in guinea pigs sensitized to the respective insulins. Neutralizing antibodies to insulin were developed in animals, which then became so resistant to insulin that their serum protected mice from insulin hypoglycemia. Endogenous insulins of guinea pig and sheep were not neutralized by homologous antibodies. Endogenous guinea pig insulin was immunologically distinct from "altered" guinea pig insulin, which the authors extracted by a different method. Various immunologic differences and similarities of insulins from different species are reported. Mice were made temporarily diabetic by the injection of guinea pig anti-insulin serum, but in relating the results to man Moloney & Coval (259) state, "It is most unlikely that immunization with endogenous insulin ever takes place." The properties of various insulins have been further studied by chromatography and by their reaction to anti-insulin (260). Ox insulin and guinea pig insulin were separated by chromatography, and the relation of chemical fractionation and neutralization by antibody of the hypoglycemic action was described. Using labelled bovine insulin, Burrows *et al.* (261) observed that in serum from insulin-resistant subjects, insulin migrated with the gamma-globulin zone upon paper electrophoresis whereas no such migration was seen in the serum from nonresistant persons. They proposed that this was caused by the binding of insulin to antibodies and, as in the studies above (259, 260), found that human insulin was not bound in this way to antibodies induced by beef insulin. Berson & Yalow (262, 263, 264) have developed their earlier work on insulin-binding antibody. Their fractionation of plasma and electrophoretic identification of the labelled insulin complexes revealed that antibody was present in alpha- and beta-globulins and sometimes in gamma-globulins. The distribution of antibody among these fractions indicates that

"complexed and uncomplexed" insulin antibodies have about the same mobility. They also observed that the inhibition of hepatic insulinase by the plasma of insulin treated subjects resulted from presence of insulin-binding antibodies (263). In short, insulin-binding antibodies protect insulin from degradation but also deprive the tissues of the action of insulin. Release of this bound insulin may account for irregularly occurring hypoglycemic reactions in subjects who at times tolerate large doses of insulin (264). Antoniadou *et al.* (265) have pursued their studies on the fractions of plasma which contain insulinlike activity by the use of elution from cationic exchange resin. Crystalline insulin, unlike the insulinlike factor of plasma, was not absorbed by the exchange resins, a fact which led them to conclude that a special binding of insulin to some plasma constituent, some kind of polymerization of human insulin, or other difference must be present. They have also summarized the whole problem of the bioassay of insulin in blood and its technical difficulties, with the addition of some results using adipose tissue of the rat (266).

LITERATURE CITED

1. Lazarow, A., *Diabetes*, **6**, 222-32 (1957)
2. Goldner, M. G., and Volk, B. W., *Ciba Foundation Colloq. on Endocrinol.*, **9**, 75-85 (1956)
3. Ferner, H., *Ciba Foundation Colloq. on Endocrinol.*, **9**, 2-13 (1956)
4. Fodden, J. H., *Am. J. Clin. Pathol.*, **23**, 994-98 (1953)
5. Fodden, J. H., *Arch. Pathol.*, **61**, 65-75 (1956)
6. Lacy, P. E., *Diabetes*, **6**, 498-507 (1957)
7. Bencosme, S. A., Mariz, S., and Frei, J., *Endocrinology*, **61**, 1-11 (1957)
8. Mosca, L., *Quart. J. Exptl. Physiol.*, **42**, 267-78 (1957)
9. Kracht, J., *Jahresber. des Tuberc.-Forsch.inst. Borstel 1954-55*, p. 181-88.
10. Best, C. H., Haist, R. E., and Wrenshall, G. A., *Ann. Rev. Physiol.*, **17**, 393-416 (1955)
11. Wrenshall, G. A., in *Newer Concepts of the Causes and Treatment of Diabetes Mellitus*, 68-82 (The National Vitamin Foundation, Inc., New York, N. Y., 181 pp., 1954)
12. Wrenshall, G. A., *Can. Med. Assoc. J.*, **74**, 871-74 (1956)
13. Foa, P. P., Galansino, G., and Pozza, G., *Recent Progr. in Hormone Research*, **13**, 473-510 (1957)
14. Schulze, W., *Ciba Foundation Colloq. on Endocrinol.*, **9**, 147-66 (1956)
15. Staub, A., Sinn, L., and Behrens, O. K., *J. Biol. Chem.*, **214**, 619-32 (1955)
16. Bromer, W. W., Sinn, L. G., Staub, A., and Behrens, O. K., *J. Am. Chem. Soc.*, **78**, 3858-60 (1956)
17. Behrens, O. K., Staub, A., Root, M. A., and Bromer, W. W., *Ciba Foundation Colloq. on Endocrinol.*, **9**, 167-78 (1956)
18. Root, M. A., Ellis, J., and Staub, A., *Proc. Soc. Exptl. Biol. Med.*, **85**, 507-11 (1954)
19. Staub, A., and Behrens, O. K., *J. Clin. Invest.*, **33**, 1629-33 (1954)
20. Sirek, O. V., Sirek, A., and Best, C. H., *Am. J. Physiol.*, **188**, 17-20 (1957)
21. Moya, F., *Endocrinology*, **56**, 312-21 (1955)
22. Cornblath, M., *Am. J. Physiol.*, **183**, 240-44 (1955)
23. Mohnike, G., *Klin. Wochschr.*, **33**, 132-33 (1955)
24. Boser, H., and Mohnike, G., *Z. ges. exptl. Med.*, **125**, 499-506 (1955)

25. Cori, G. T., and Cori, C. F., *J. Biol. Chem.*, **158**, 321-32 (1945)
26. Sutherland, E. W., Wosilait, W. D., and Rall, T. W., *Ciba Foundation Colloq. on Endocrinol.*, **9**, 179-91 (1956)
27. Raben, M. S., *Recent Prog. in Hormone Research*, **8**, 471 (1953)
28. Ui, M., Kobayashi, B., and Ito, Y., *Endocrinol. Japon.*, **3**, 191-96 (1956)
29. Lazarus, S. S., Volk, B. W., and Goldner, M. G., *Metabolism, Clin. and Exptl.*, **3**, 449-55 (1954)
30. Planas, J., and Luch, M., *Rev. españ. fisiol.*, **12**, 295-300 (1956)
31. Vuylsteke, C. A., Cornelis, G., and de Duve, C., *Arch. intern. physiol.*, **60**, 128-31 (1952)
32. Bencosme, S. A., Liepa, E., and Lazarus, S. S., *Proc. Soc. Exptl. Biol. Med.*, **90**, 387-92 (1955)
33. Elrick, H., Staub, A., and Maske, H., *New Engl. J. Med.*, **256**, 742-47 (1957)
34. Bierich, J. R., and Kornatz-Stegman, B., *Monatsschr. Kinderheilk.*, **102**, 49-51 (1954)
35. McQuarrie, I., *Am. J. Diseases Children*, **87**, 399-428 (1954)
36. Mirsky, I. A., Futterman, P., Wachman, J., and Perisutti, G., *Endocrinology*, **49**, 73-81 (1951)
37. Marks, H. P., and Young, F. G., *J. Endocrinol.*, **1**, 470-510 (1939)
38. Singh, I., *Lancet*, **I**, 422-25 (1955)
39. Elrick, H., Rachiele, F. J., and Hlad, C. J., Jr., *Diabetes*, **7**, 129-32 (1958)
40. Salter, J. M., Davidson, I. W. F., and Best, C. H., *Diabetes*, **6**, 248-52 (1957)
41. Goldner, M. G., Jauregui, R. H., and Weisenfeld, S., *Am. J. Physiol.*, **179**, 25-28 (1954)
42. Jauregui, R. H., *Medicina (Mex.)*, **36**, 503-16 (1956)
43. Tomizawa, H. H., and Williams, R. H., *J. Biol. Chem.*, **217**, 685-94 (1955)
44. Tomizawa, H. H., Tyberghein, J. M., Halsey, Y. D., and Williams, R. H., *Federation Proc.*, **15**, 534 (1956)
45. Foa, P. P., *Advances in Internal Med.*, **6**, 29-58 (1954)
46. Cahill, G. F., Jr., Zottu, S., and Earle, A. S., *Endocrinology*, **60**, 265-69 (1957)
47. Costa, E., Galansino, G., Pozza, G., and Foa, P. P., *Proc. Soc. Exptl. Biol. Med.*, **91**, 574-76 (1956)
48. Craig, A. B., Jr., *Am. J. Physiol.*, **193**, 425-30 (1958)
49. Galansino, G., Weinstein, H. R., Magill, A. M., and Foa, P. P., *Am. J. Physiol.*, **180**, 27-30 (1955)
50. Root, M. A., *Proc. Soc. Exptl. Biol. Med.*, **87**, 108-10 (1954)
51. Rall, T. W., Sutherland, E. W., and Berthet, J., *J. Biol. Chem.*, **224**, 463-75 (1957)
52. Rall, T. W., Sutherland, E. W., and Wosilait, W. D., *J. Biol. Chem.*, **218**, 483-95 (1956)
53. Sutherland, E. W., and Wosilait, W. D., *J. Biol. Chem.*, **218**, 459-68 (1956)
54. Wosilait, W. D., and Sutherland, E. W., *J. Biol. Chem.*, **218**, 469-81 (1956)
55. Tomizawa, H. H., and Hyde, P. M., *Am. J. Physiol.*, **193**, 52-54 (1958)
56. Arteta, J. L., and Carballido, A., *J. Endocrinol.*, **15**, 243-47 (1957)
57. Vuylsteke, C. A., de Duve, C., and Nys, A., *Arch. intern. physiol.*, **57**, 445-46 (1950)
58. Costa, E., Galansino, G., Pozza, G., and Foa, P. P., *Proc. Soc. Exptl. Biol. Med.*, **91**, 574-76 (1956)
59. Kirtley, W. R., Waife, S. O., and Peck, F. B., *Proc. Soc. Exptl. Biol. Med.*, **83**, 387-89 (1953)
60. Weisenfeld, S., *Proc. Soc. Exptl. Biol. Med.*, **97**, 764-67 (1958)

61. Barrett, A. M., and Sayers, G., *Endocrinology*, **62**, 637-45 (1958)
62. Stunkard, A. J., Van Itallie, T. B., and Reiss, B., *Proc. Soc. Exptl. Biol. Med.*, **89**, 258-61 (1955)
63. Robinson, R. M., Harris, K., Hlad, C. J., and Eiseman, B., *Proc. Soc. Exptl. Biol. Med.*, **96**, 518-20 (1957)
64. Elrick, H., Witten, T. A., and Arai, Y., *New Engl. J. Med.*, **258**, 476-80 (1958)
65. Schulman, J. L., and Greben, S. E., *J. Clin. Invest.*, **36**, 74-80 (1957)
66. Foa, P. P., *Ciba Foundation Colloq. on Endocrinol.*, **9**, 55-71 (1956)
67. Whitney, J. E., and Young, F. G., *Biochem. J.*, **66**, 645-48 (1957)
68. Candela, J. L. R., Rovera, J., and Candela, R. R., *Medicina (Madrid)*, **22**, 167-72 (1954)
69. Pozza, G., Galansino, G., Hoffeld, H., and Foa, P. P., *Am. J. Physiol.*, **192**, 497-500 (1958)
70. Leites, S. M., Pavlov, G. T., and Yakusheva, G. S., *Fiziol. Z. S.S.S.R.*, **41**, 249-56 (1955)
71. Veller, N. S., Genes, S. G., Radkina, B. S., and Charnaya, P. M., *Problemy Endokrinol. i Gormonoterap.*, **1**, 77-84 (1955)
72. Link, R. P., *Am. J. Vet. Research*, **14**, 150-59 (1953)
73. Yoshikawa, K., *Tōhoku Igaku Zasshi*, **49**, 25 (1954)
74. Treadwell, C. R., and Roe, J. H., Jr., *Proc. Soc. Exptl. Biol. Med.*, **86**, 878-81 (1954)
75. Scow, R. O., *Endocrinology*, **60**, 359-67 (1957)
76. Scow, R. O., Wagner, E. M., and Cardeza, A., *Endocrinology*, **61**, 380-91 (1957)
77. Okamoto, K., *Tōhoku J. Exptl. Med.*, **61**, Suppl. III, 1-116 (1955)
78. Rowntree, L. G., Clark, J. H., and Hanson, A. M., *J. Am. Med. Assoc.*, **103**, 1425-30 (1934)
79. Beach, E. F., Cullimore, O. S., and Bradshaw, P. J., *Am. J. Physiol.*, **191**, 19-22 (1957)
80. MacDonald, M. K., and Bhattacharya, S. K., *Quart. J. Exptl. Physiol.*, **41**, 153-61 (1956)
81. Haist, R. E., in *Hypophyseal Growth Hormone; Nature and Actions*, 437-47 (Smith, R. W., Gaebler, O. H., and Long, C. N. H., Eds. McGraw-Hill Book Co., Inc., New York, N. Y., 576 pp., 1955)
82. Kinash, B., and Haist, R. E., *Can. J. Biochem. and Physiol.*, **33**, 380-84 (1955)
83. Wrenshall, G. A., Best, C. H., and Hartroft, W. S., *Can. J. Biochem. and Physiol.*, **35**, 527-35 (1957)
84. Lahnschagre, C. J., Haessig, B. K., and Wrenshall, G. A., *Can. J. Biochem. and Physiol.*, **35**, 537-43 (1957)
85. Wrenshall, G. A., Casselman, W. G. B., and Best, C. H., *Can. J. Biochem. and Physiol.*, **35**, 545-48 (1957)
86. Abrams, G. D., Baker, B. L., Ingle, D. J., and Li, C. H., *Endocrinology*, **53**, 252-60 (1953)
87. Batts, A. A., Bennett, L. L., Ellis, S., and George, R. A., *Endocrinology*, **59**, 620-30 (1956)
88. Chaikof, L., and Campbell, J., *Endocrinology*, **61**, 618-26 (1957)
89. Dohan, F. C., and Lukens, F. D. W., *Proc. Soc. Exptl. Biol. Med.*, **42**, 167-71 (1939)
90. Bennett, L. L., in *Hypophyseal Growth Hormone; Nature and Actions*, 447-53 (Smith, R. W., Gaebler, O. H., and Long, C. N. H., Eds. McGraw-Hill Book Co., Inc., New York, N. Y., 576 pp., 1955)

91. Altszuler, N., Steele, R., Wall, J. S., and de Bodo, R. C., *Proc. Soc. Exptl. Biol. Med.*, **94**, 744-46 (1957)
92. Randle, P. J., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 115-31 (1957)
93. Lazarus, S. S., and Volk, B. W., *Diabetes*, **7**, 15-20 (1958)
94. Volk, B. W., and Lazarus, S. S., *Am. J. Pathol.*, **34**, 121-36 (1958)
95. Buse, J., Gundersen, K., and Lukens, F. D. W., *Diabetes*, **6**, 428-32 (1957)
96. Bastenie, P., *Cortico-surrenale et diabete humain* (Masson et Cie., Paris, France, 506 pp., 1956)
97. Ketterer, B., Randle, P. J., and Young, F. G., *Ergeb. Physiol. u. exptl. Pharmacol.*, **49**, 128-211 (1957)
98. Editorial, *Lancet*, **II**, 1210 (1957)
99. de Bodo, R. C., and Altszuler, N., *Vitamins and Hormones*, **15**, 205-58 (1957)
100. Houssay, B. A., *Tercer Congr. Panam. de Endocrinol.*, *Santiago*, **I**, 15-30 (1954)
101. de Bodo, R. C., and Sinkoff, M. W., *Diabetes*, **3**, 87-93 (1954)
102. Sinkoff, M. W., de Bodo, R. C., Den, H., and Kiang, S. P., *Am. J. Physiol.*, **176**, 361-66 (1954)
103. de Bodo, R. C., and Altszuler, N., in *The Hypophyseal Growth Hormone, Nature and Actions* (Smith, R. W., Jr., Gaebler, O. H., and Long, C. N. H., Eds., McGraw-Hill Book Co., Inc., New York, N. Y., 576 pp., 1955)
104. de Bodo, R. C., and Altszuler, N., *Diabetes*, **5**, 194-202 (1956)
105. Knobil, E., Wolf, R. C., Greep, R. O., and Wilhelmi, A. E., *Endocrinology*, **60**, 166-68 (1957)
106. Beck, J. C., McGarry, E. E., Dyrenfurth, I., and Venning, E. H., *Science*, **125**, 184-85 (1957)
107. Volk, B. W., Lazarus, S. S., and Lew, M., *Metabolism*, **4**, 10-18 (1955)
108. Steele, R., Wall, J. S., de Bodo, R. C., and Altszuler, N., *Am. J. Physiol.*, **187**, 15-24 (1956)
109. Steele, R., Wall, J. S., de Bodo, R. C., and Altszuler, N., *Am. J. Physiol.*, **187**, 25-31 (1956)
110. Wall, J. S., Steele, R., de Bodo, R. C., and Altszuler, N., *Am. J. Physiol.*, **189**, 43-50 (1957)
111. Wall, J. S., Steele, R., de Bodo, R. C., and Altszuler, N., *Am. J. Physiol.*, **189**, 51-56 (1957)
112. Altszuler, N., Steele, R., Wall, J. S., and de Bodo, R. C., *Am. J. Physiol.*, **191**, 29-33 (1957)
113. Altszuler, N., Steele, R., Wall, J. S., and de Bodo, R. C., *Am. J. Physiol.*, **192**, 219-26 (1958)
114. Houssay, B. A., and Penhos, J. C., *Endocrinology*, **59**, 637-41 (1956)
115. Campbell, J., Hausler, H. R., Munroe, J. S., and Davidson, I. W. F., *Endocrinology*, **53**, 134-62 (1953)
116. Warner, R. C., Weber, I., de Bodo, R. C., and Kurtz, M., *Am. J. Physiol.*, **190**, 121-28 (1957)
117. Stevenson, O. R., Coulson, R. A., and Hernandez, T., *Am. J. Physiol.*, **191**, 95-102 (1957)
118. Thorn, G. W., Renold, A. E., and Winegrad, A. I., *Brit. Med. J.*, **II**, 1009-17 (1957)
119. Froesch, R., *Schweiz. med. Wochschr.*, **85**, 121-27 (1955)
120. Ingle, D. J., Beary, D. F., and Purmalis, A., *Endocrinology*, **53**, 221-25 (1953)
121. Penhos, J. C., and Cardeza, A. F., *Rev. soc. arg. biol.*, **32**, 1-11 (1956)
122. Hausberger, F. X., and Hausberger, B. C., *Am. J. Physiol.*, **183**, 302-6 (1955)
123. Brown, J. H. U., *Endocrinology*, **53**, 116-18 (1953)

124. McArthur, J. W., et al., *J. Clin. Invest.*, **33**, 420-35 (1954)
125. McArthur, J. W., et al., *J. Clin. Invest.*, **33**, 437-49 (1954)
126. Bloom, W. L., and Russell, J. A., *Am. J. Physiol.*, **183**, 356-64 (1955)
127. Engel, F. L., Fredericks, J., and Cole, B. T., *Endocrinology*, **60**, 446-59 (1957)
128. Ries, N., and Allegretti, N., *Arch. intern. physiol. et biochim.*, **65**, 439-46 (1957)
129. Maske, H., *Diabetes*, **6**, 335-41 (1957)
130. Homan, J. D. H., Bouman, J., Matthijsen, R., Maske, H., and Munk, K., *Acta Physiol. et Pharmacol. Neerl.*, **5**, 255-56 (1956)
131. Landau, B. R., and Renold, A. E., *Diabetes*, **3**, 47-50 (1954)
132. Lacy, P. E., and Davies, J., *Diabetes*, **6**, 354-57 (1957)
133. Lukens, F. D. W., and Dyer, W. W., *Am. J. Med. Sci.*, **231**, 313-19 (1956)
134. Lukens, F. D. W., and Dyer, W. W., *Trans. Am. Clin. Climatol. Assoc.*, **67**, 1-8 (1955)
135. Sturtevant, F. M., Calvin, L. D., and Fuller, N. E., *Metabolism, Clin. and Exptl.*, **3**, 262-67 (1954)
136. Lukens, F. D. W., *Am. J. Med.*, **19**, 790-97 (1955)
137. Hartroft, W. S., and Wrenshall, G. A., *Diabetes*, **4**, 1-7 (1955)
138. Wrenshall, G. A., and Bogoch, A., *J. Clin. Endocrinol. and Metabolism*, **15**, 435-41 (1955)
139. Burton, T. Y., Kearns, T. P., and Rynearson, E. H., *Proc. Staff Meetings Mayo Clinic*, **32**, 735-39 (1957)
140. Everson, T. C., and Cole, W. H., *Surgery*, **37**, 260-62 (1955)
141. Sudak, F. N., Beaser, S. B., Shulman, M. H., and Fulton, G. P., *Endocrinology*, **62**, 679-82 (1958)
142. Rae, M. V., and Lewis, R. C., *Can. Med. Assoc. J.*, **77**, 691-94 (1957)
143. Eder, M., *Beitr. path. Anat. u. allgem. Pathol.*, **115**, 157-77 (1955)
144. Symposium in *Ann. N. Y. Acad. Sci.*, **71**, 1-292 (1957)
145. Symposium in *Can. Med. Assoc. J.*, **74**, 957-98 (1956)
146. Symposium in *Diabetes*, **6**, 259-89 (1957)
147. Symposium in *Metabolism, Clin. and Exptl.*, **5**, 721-977 (1956)
148. Levine, R., and Sobel, G. W., *Diabetes*, **6**, 263-69 (1957)
149. Houssay, B. A., and Penhos, J. C., *Metabolism, Clin. and Exptl.*, **5**, 727-32 (1956)
150. Houssay, B. A., and Penhos, J. C., *Rev. soc. arg. biol.*, **32**, 55-65 (1956)
151. Gordon, M. F., Buse, J. F., and Lukens, F. D. W., *Diabetes*, **6**, 7-12 (1957)
152. Houssay, B. A., Penhos, J. C., Migliorni, R. H., and Bowkett, J. R., *Rev. soc. arg. biol.*, **32**, 87-94 (1956)
153. Pozza, G., Galansino, G., and Foa, P. P., *Proc. Soc. Exptl. Biol. Med.*, **93**, 539-42 (1956)
154. Sobel, G. W., Rodriguez-Inigo, J., Morton, J. V., and Levine, R., *Metabolism, Clin. and Exptl.*, **7**, 222-26 (1958)
155. Colwell, A. R., Jr., Colwell, J. A., and Colwell, A. R., Sr., *Metabolism, Clin. and Exptl.*, **5**, 749-56 (1956)
156. Volk, B. W., and Lazarus, S. S., *Diabetes*, **7**, 125-28 (1958)
157. Lazarus, S. S., and Volk, B. W., *Endocrinology*, **62**, 292-307 (1958)
158. Unger, R. H., and Madison, L. L., *J. Clin. Invest.*, **37**, 627-30 (1958)
159. Holt, C. von, Holt, L. von, Kracht, J., Kröner, B., and Kühnau, J., *Science*, **125**, 735-36 (1957)
160. Candela, J. L. R., and Candela, R. R., *Rev. Ibérica endocrinol.*, **4**, 413-15 (1957)
161. Renold, A. E., Winegrad, A. I., Froesch, E. R., and Thorn, G. W., *Ann. N. Y. Acad. Sci.*, **71**, 71-80 (1957)
162. Candela, J. L. R., and Candela, R. R., *Rev. Ibérica endocrinol.*, **4**, 309-11 (1957)

163. Houssay, B. A., and Migliorini, R. H., *Rev. soc. arg. biol.*, **32**, 94-99 (1956)
164. Caren, R., and Corbo, L., *J. Clin. Invest.*, **36**, 1546-50 (1957)
165. Candela, J. L. R., and Candela, R. R., *Rev. Ibérica endocrinol.*, **4**, 409-11 (1957)
166. Ashmore, J., Cahill, G. F., Jr., Earle, A. S., and Zottu, S., *Diabetes*, **7**, 1-8 (1958)
167. Craig, J. W., and Miller, M., *Diabetes*, **6**, 280-87 (1957)
168. Segal, S., Frawley, T. F., and Foley, J., *Diabetes*, **6**, 422-25 (1957)
169. Stadie, W. C., *Diabetes*, **7**, 61-63 (1958)
170. Sanger, F., *Ciba Foundation Colloq. on Endocrinol.*, **9**, 110-19 (1956)
171. Sanger, F., in *Currents in Biochemical Research*, 434-59 (David E. Green, Ed., Interscience Publishers, Inc., N. Y., 697 pp., 1956)
172. Fredericq, E., *Ciba Foundation Colloq. on Endocrinol.*, **9**, 89-103 (1956)
173. Waugh, D. F., *Ciba Foundation Colloq. on Endocrinol.*, **9**, 122-32 (1956)
174. Hodgkin, D. C., and Oughton, B., *Ciba Foundation Colloq. on Endocrinol.*, **9**, 133-41 (1956)
175. Levine, R., and Goldstein, M. S., *Recent Progr. in Hormone Research*, **11**, 343-75 (1955)
176. Stadie, W. C., *Physiol. Revs.*, **34**, 52-100 (1954)
177. Stadie, W. C., *Diabetes*, **5**, 263-75 (1956)
178. Stadie, W. C., *Bull. N. Y. Acad. Med.*, **34**, 5-20 (1958)
179. Stadie, W. C., *Trans. & Studies Coll. Physicians Phila.*, **25**, 133-43 (1958)
180. Park, C. R., in *Hypophyseal Growth Hormone; Nature and Actions*, 394-405 (Smith, R. W., Gaebler, O. H., and Long, C. N. H., Eds., McGraw-Hill Book Co., Inc., New York, N. Y., 576 pp., 1955)
181. Krahle, M. E., *Perspectives in Biol. and Med.*, **1**, 69-96 (1957)
182. Renold, A. E., Ashmore, J., and Hastings, A. B., *Vitamins and Hormones*, **14**, 140-85 (1956)
183. Ross, E. J., *Medicine*, **35**, 355-88 (1956)
184. Danowski, T. S., *Diabetes Mellitus, with Emphasis on Children and Young Adults* (Williams & Wilkins Co., Baltimore, Md., 510 pp., 1957)
185. Kinsell, L. W., Editor, *Hormonal Regulation of Energy Metabolism* (Charles C Thomas, Publisher, Springfield, Ill., 242 pp., 1957)
186. Stetten, D., Jr., and Bloom, B., in *The Hormones*, 175-99 (Pincus, G., and Thimann, K. V., Eds., Vol. III, Academic Press, Inc., New York, N. Y., 1012 pp., 1955)
187. McIlwain, H., *Biochemistry and the Central Nervous System* (J. & A. Churchill, Ltd., London, Engl., 272 pp., 1955)
188. Geiger, A., and Yamasaki, S., *J. Neurochem.*, **1**, 93-100 (1956)
189. Elgee, N. J., Williams, R. H., and Lee, N. D., *J. Clin. Invest.*, **33**, 1252-60 (1954)
190. Park, C. R., Johnson, L. H., Wright, J. H., Jr., and Batsel, H., *Am. J. Physiol.*, **191**, 13-18 (1957)
191. Russell, J. A., and Bloom, W., *Endocrinology*, **58**, 83-94 (1956)
192. Lukens, F. D. W., *Am. J. Physiol.*, **192**, 485-90 (1958)
193. Fisher, R. B., and Lindsay, D. B., *J. Physiol. (London)*, **131**, 526-41 (1956)
194. Sacks, J., and Bakshy, S., *Am. J. Physiol.*, **189**, 339-42 (1957)
195. Gourley, D. R. H., *Am. J. Physiol.*, **189**, 489-94 (1957)
196. Fritz, I. B., Shattton, J., Morton, J. V., and Levine, R., *Am. J. Physiol.*, **189**, 57-62 (1957)
197. Dunn, D. F., Friedman, B., Maass, A. R., Reichard, G. A., and Weinhouse, S., *J. Biol. Chem.*, **225**, 225-37 (1957)

198. Henderson, M. J., Wrenshall, G. A., and Odense, P., *Can. J. Biochem. and Physiol.*, **33**, 926 (1955)
199. Bondy, P. K., Bloom, W. L., Whitner, V. S., and Farrar, B. W., *J. Clin. Invest.*, **28**, 1126-33 (1949)
200. Bearn, A. G., Billing, B. H., and Sherlock, S., *Clin. Sci.*, **11**, 151-65 (1952)
201. Segal, S., Wyngaarden, J. B., and Foley, J., *J. Clin. Invest.*, **36**, 1383-1407 (1957)
202. Beloff-Chain, A., Catanzaro, R., Chain, E. B., Masi, I., Pocchiari, F., and Rossi, C., *Proc. Roy. Soc. (London)*, [B]**143**, 481-503 (1955)
203. Lobeck, C. C., and Forbes, G. B., *Metabolism, Clin. and Exptl.*, **7**, 133-40 (1958)
204. Shaw, W. N., and Stadie, W. C., *J. Biol. Chem.*, **227**, 115-34 (1957)
205. Hopkinson, L., and Kerly, M., *J. Physiol. (London)*, **128**, 113-21 (1955)
206. Ingle, D. J., Torralba, G., and Flores, V., *Proc. Soc. Exptl. Biol. Med.*, **89**, 625-26 (1955)
207. Goldstein, M. S., Mullick, V., Huddlestun, B., and Levine, R., *Am. J. Physiol.*, **173**, 212-16 (1953)
208. Joslin, E. P., Root, H. F., White, P., and Marble, A., *Treatment of Diabetes Mellitus*, Ninth Ed. (Lea and Febiger, Philadelphia, Pa., 771 pp., 1952)
209. Richardson, R., *J. Clin. Invest.*, **13**, 949-61 (1934)
210. Issekutz, B., *Federation Proc.*, **17**, 380 (1958)
211. Madison, L. L., and Unger, R. H., *J. Clin. Invest.*, **37**, 631-39 (1958)
212. Haft, D. E., and Miller, L. L., *Am. J. Physiol.*, **192**, 33-42 (1958)
213. Renold, A. E., Hastings, A. B., Nesbitt, F. B., and Ashmore, J., *J. Biol. Chem.*, **213**, 135-46 (1955)
214. Ashmore, J., Cahill, G. F., Jr., Hastings, A. B., and Zottu, S., *J. Biol. Chem.*, **224**, 225-35 (1957)
215. Cahill, G. F., Jr., Ashmore, J., Earle, A. S., and Zottu, S., *Am. J. Physiol.*, **192**, 491-96 (1958)
216. Felts, J. M., Doell, R. G., and Chaikoff, I. L., *J. Biol. Chem.*, **219**, 473-78 (1956)
217. Lossow, W. J., Brown, G. W., Jr., and Chaikoff, I. L., *J. Biol. Chem.*, **220**, 839-49 (1956)
218. Bauman, J. W., Hill, R., and Chaikoff, I. L., *Endocrinology*, **60**, 514-18 (1957)
219. Beaton, G. H., and Curry, D. M., *Endocrinology*, **58**, 797-801 (1956)
220. Stetten, D., Jr., *Diabetes*, **6**, 391-401 (1957)
221. Cagan, R. N., Sobel, A. E., Nichols, R. A., and Loewe, L., *Metabolism, Clin. and Exptl.*, **3**, 168-72 (1954)
222. Bodur, H., and Favarger, P., *Helv. Physiol. et Pharmacol. Acta*, **15**, 345-52 (1957)
223. Itzhaki, S., and Wertheimer, E., *Endocrinology*, **61**, 72-78 (1957)
224. Renold, A. E., Winegrad, A. I., and Martin, D. B., *Helv. Med. Acta*, **24**, 322-27 (1957)
225. Renold, A. E., *Diabetes*, **7**, 219-20 (1958)
226. Lawrence, R. D., *Ann. Internal Med.*, **43**, 1199-1208 (1955)
227. Krah, M. E., *Recent Progr. in Hormone Research*, **12**, 199-219 (1956)
228. Munro, H. N., *Scot. Med. J.*, **1**, 285-93 (1956)
229. Lukens, F. D. W., *Diabetes*, **2**, 491-97 (1953)
230. Sinex, F. M., MacMullen, J., and Hastings, A. B., *J. Biol. Chem.*, **198**, 615-19 (1952)
231. Prudden, J. F., Young, M. K., and Stirman, J. A., *J. Lab. Clin. Med.*, **47**, 370-85 (1956)

232. Lukens, F. D. W., and McCann, S. M., in *The Hypophyseal Growth Hormone, Nature and Actions*, 225-34 (Smith, R. W., Gaebler, O. H., and Long, C. N. H., Eds., McGraw-Hill Book Co., Inc., New York, N. Y., 576 pp., 1955)
233. Salter, J., and Best, C. H., *Brit. Med. J.*, **II**, 353-56 (1953)
234. Lawrence, R. T. B., Salter, J. M., and Best, C. H., *Brit. Med. J.*, **II**, 437-39 (1954)
235. Wagner, E. M., and Scow, R. O., *Endocrinology*, **61**, 419-25 (1957)
236. Scow, R. O., Wagner, E. M., and Ronov, E., *Endocrinology*, **62**, 593-604 (1958)
237. Mirsky, I. A., *Recent Progr. in Hormone Research*, **13**, 429-64 (1957)
238. Elgee, N. J., Bailey, R. E., and Williams, R. H., *Proc. Soc. Exptl. Biol. Med.*, **88**, 110-13 (1955)
239. Elgee, N. J., and Williams, R. H., *Diabetes*, **4**, 87-90 (1955)
240. Elgee, N. J., and Williams, R. H., *Proc. Soc. Exptl. Biol. Med.*, **87**, 352-55 (1954)
241. Weisenfeld, S., Jauregui, R. H., and Goldner, M. G., *Am. J. Physiol.*, **188**, 45-48 (1957)
242. Mirsky, I. A., Perisutti, G., and Dixon, F. J., *J. Biol. Chem.*, **214**, 397-408 (1955)
243. Tomizawa, H. H., Nutley, M. L., Narahara, H. T., and Williams, R. H., *J. Biol. Chem.*, **214**, 285-94 (1955)
244. Narahara, H. T., Tomizawa, H. H., Miller, R., and Williams, R. H., *J. Biol. Chem.*, **217**, 675-84 (1955)
245. Elgee, N. J., and Williams, R. H., *Am. J. Physiol.*, **180**, 13-15 (1955)
246. Elgee, N. J., and Williams, R. H., *Am. J. Physiol.*, **180**, 9-12 (1955)
247. Elgee, N. J., and Williams, R. H., *Diabetes*, **4**, 8-12 (1955)
248. Drury, D. R., Karasek, M. A., Britton, B., and Wick, A. N., *Am. J. Physiol.*, **192**, 501-5 (1958)
249. Wick, A. N., and Drury, D. R., *Proc. Soc. Exptl. Biol. Med.*, **97**, 514-16 (1958)
250. Vallance-Owen, J., *Diabetes*, **5**, 248-50 (1956)
251. Randle, P. J., in *Hypophyseal Growth Hormone; Nature and Actions*, 413-36 (Smith, R. W., Gaebler, O. H., and Long, C. N. H., Eds., McGraw-Hill Book Co., Inc., New York, N. Y., 576 pp., 1955)
252. Christophe, J., *Acta gastroenterol. Belg.*, **17**, 787-98 (1954)
253. Loraine, J. A., *The Clinical Application of Hormone Assay* (E. & S. Livingstone, Ltd., Edinburgh, Scotland, 368 pp., 1958)
254. Vallance-Owen, J., and Lukens, F. D. W., *Endocrinology*, **60**, 625-33 (1957)
255. Whitney, J. E., and Young, F. G., *Biochem. J.*, **66**, 648-51 (1957)
256. Field, J. B., and Stetten, D., Jr., *Diabetes*, **5**, 391-96 (1956)
257. Field, J. B., Tietze, F., and Stetten, D., Jr., *J. Clin. Invest.*, **36**, 1588-93 (1957)
258. Willebrands, A. F., van der Geld, H., and Groen, J., *Diabetes*, **7**, 119-24 (1958)
259. Moloney, P. J., and Coval, M., *Biochem. J.*, **59**, 179-85 (1955)
260. Goldsmith, L., and Moloney, P. J., *Biochem. J.*, **66**, 432-34 (1957)
261. Burrows, B. A., Peters, T., and Lowell, F. C., *J. Clin. Invest.*, **36**, 393-97 (1957)
262. Berson, S. A., and Yalow, R. S., *J. Clin. Invest.*, **36**, 642-47 (1957)
263. Yalow, R. S., and Berson, S. A., *J. Clin. Invest.*, **36**, 648-55 (1957)
264. Berson, S. A., and Yalow, R. S., *Diabetes*, **6**, 402-7 (1957)
265. Antoniadis, H. N., Beigelman, P. M., Pennell, R. B., Thorn, G. W., and Oncley, J. L., *Metabolism, Clin. and Exptl.*, **7**, 266-68 (1958)
266. Beigelman, P. M., and Antoniadis, H. N., *Metabolism, Clin. and Exptl.*, **7**, 269-73 (1958)

RECENT RUSSIAN RESEARCH IN METABOLISM AND ENDOCRINOLOGY¹

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INTRODUCTION

Soviet physiologists and biochemists, like most Russian workers in the biological sciences, are strongly under the influence of the Pavlov tradition. This gives to their work a certain unity, both in theoretical and practical aspects, and serves to tie together studies on metabolism and on endocrinology. The unifying principle is the Pavlovian idea that the activities of the central nervous system, especially the cerebral cortex, control almost all processes which occur in the body. This control may be exerted directly through the nervous system or indirectly through hormonal influences. Both types of control exert their effects through changes in the metabolic state of the body. In turn, factors which act directly on metabolic reactions or on the endocrine organs may alter brain metabolism and thus change many other processes [Genes (1); Aleshin (2)]. These ideas are, of course, not confined to Soviet biologists, but probably nowhere else have they had so much influence in directing the course of research, although cautioning voices have occasionally been raised even in the Union of Soviet Socialist Republics to warn against a too rigid interpretation of the Pavlov concepts [Speranskaya (3)].

In conformity with the central ideas thus described, much attention has been directed toward protein metabolism and especially to the factors which govern regeneration of proteins in various organs and tissues. The problem of the energetics of protein metabolism has produced extensive studies of carbohydrate metabolism. Relatively little activity is found in the field of lipid metabolism. Endocrine activity is, of course, intimately associated with metabolism; and, aside from purely clinical studies which are not considered in this paper, most of the endocrinological research has been directed toward establishing the action of hormones on metabolism as controlled by the nervous system, and the reciprocal action of the hormones on the nervous system itself. It is, therefore, natural that much work has been done on the metabolism of the brain. Finally, in recent years, considerable attention has been devoted to the effects of ionizing radiation on metabolic states.

In experimental work the influence of Pavlov is also strong, and the conditioned reflex is much used as a tool in the laboratory. Stimulation of the central nervous system and of various organs by conditioned reflexes is

¹ The survey of the literature was concluded May 15, 1958. Since few Russian journals for 1958 had been received by this date, the period covered by this review is actually from 1953 to 1957, with some scattered early references in 1958 also included.

greatly favored as a means of studying metabolic processes. The widespread clinical use of "therapeutic sleep" under relatively mild, but prolonged, narcosis has led to its frequent use in the laboratory when inhibition of some activity of the central nervous system is desired. These theoretical ideas and practical procedures have been very characteristic of Soviet studies on metabolism and endocrinology in the years from 1953 to 1958, and most of the work reviewed in this paper will be related to such studies. Other phases of Soviet investigations will be mentioned only incidentally.

It has been considered desirable to review a rather large number of papers, with the aim of indicating the major trends in Russian research, rather than to give detailed accounts of individual investigations. Fuller accounts of some of the studies reported here have been given by Stekol (4). The amount of space which has been devoted to the various topics in the present review is a rough measure of the amount of activity in the field discussed.

METABOLISM

Protein metabolism.—The importance of proteins in carrying out the major functions of the body is fully recognized, and many studies of all phases of protein metabolism have been carried out. From the absorption and utilization of amino acids, attention has shifted to problems of formation and renewal of different proteins by various organs and under various conditions.

The importance of phosphorylation reactions among the proteins has led to many studies of this reaction, especially because of the availability of P³². Intestinal absorption of amino acids involves their phosphorylation, the rate depending on the individual acid [Shishova (5)]. Reactions of amino acids in the tissues depend largely on transaminations and an optimum amount of vitamin B₆. Alanine is synthesized in the liver either by indirect amination with the aid of glutamic dehydrogenase and transaminase, or by transamination alone [Braunshtein & Azarkh (6)]. Normally there is a high residual activity of transaminase even in organs deficient in the B complex [Efimochkina *et al.* (7)]. Pyridoxine is also essential in transsulfuration reactions, which occur to a varying extent in different tissues. Brain and skeletal muscle tissues lack transsulfurases and so cannot form cysteine by transfer of sulfur from methionine to serine. Kidney and spleen are the most efficient organs for this reaction [Goryachenkova (8)]. The incorporation of sulfur from cystine into tissue proteins also requires the presence of B₆ in transsulfurases. Competition between transaminases and transsulfurases for B₆ has been observed [Volovnik (9)].

Vitamin A is also concerned in sulfur metabolism, since it inhibits conversion of cysteine into cystine and the oxidation of sulphydryl groups. This inhibition prevents excessive keratinization, which results from cross-linking of newly formed disulfide bonds [Balakhovskii & Drozdova (10)]. Vitamin A-deficient rats excrete more sulfur as sulfate than do normal animals, indicating that all oxidations of sulfur compounds are inhibited by Vitamin A [Balakhovskii & Kuznetsova (11)].

Nicotinic acid is closely connected with cystine metabolism. The harmful effects of excess cystine in the diet can be avoided by adding more nicotinic acid or its precursor, tryptophan [Cherkes *et al.* (12); Dinerman (13)]. There is a direct relation between dietary methionine and cystine and the available nicotinic acid, showing that it is needed for the metabolism of these compounds [Cherkes & Fil'chgin (14)].

Tryptophan is an important part of many glycoproteins, since combination of proteins with polysaccharides occurs through this amino acid. Tyrosine is less important in such combinations [Plyshevskaya & Rozenfel'd (15)]. The distribution of tryptophan in various organs varies [Yushkevich & Kedrovskii (16)], though the content of analogous organs of mammals and amphibia is similar [Kedrovskii & Yushkevich (17)]. These facts indicate a specific function for this amino acid.

It is well established that the amino acids are used to build up proteins in the body. The claim that labeled amino acids are incorporated into isolated proteinlike compounds *in vitro* has been made several times [Konikova *et al.* (18); Samarina *et al.* (19); Kritsman *et al.* (20)], but the experimental procedures used have been criticized by other investigators on the ground that the preparations were contaminated by bacteria. The latter workers conclude that protein has no inherent ability for the self-renewal of its amino acids in the absence of enzyme systems [Orekhovich *et al.* (21); Medvedev (22)].

Most of the studies on formation of proteins have been made with the aid of radioactive isotopes, usually P^{32} or S^{35} . Savitskii & Leshchinskii (23) studied the uptake of radioactive phosphorus by serum proteins after severe bleeding in rabbits and found that when doses of $1.5 \mu\text{c./kg.}$ were given, regeneration of the proteins, particularly fibrinogen, was increased, while doses of 0.1 mc./kg. decreased protein formation. While this study indicates that some caution should be used in interpreting results of investigations on proteins with radioactive isotopes, the majority of such investigations have not taken account of this fact.

Not only amino acids are used in the synthesis of proteins. Transpeptidation is a factor [Kazanova & Arekhovich (24)], and serum proteins can be incorporated directly into liver proteins [Kuzovleva (25)] and antibodies [Uchitel & Konikova (26)]. Structural changes in proteins produced by urea can increase the rate of incorporation of labeled methionine into the tissues at the site of a wound [Zamanskii *et al.* (27)].

Soviet biologists have been much interested in the processes occurring in ontogenesis. There are a number of comparative studies of the stages of development at which specific proteins appear. The contractile proteins of skeletal muscle are low during the embryonic period and increase in the early postnatal period [Kasavina (28); Ivanov *et al.* (29)]. Animals which are relatively mature at birth, such as guinea pigs or chickens, have a higher concentration of aldolase and glyceraldehyde dehydrogenase in their muscles at birth than do less mature animals such as rats and rabbits. The latter animals gradually accumulate these sarcoplasm proteins as they mature

[Kasavina & Torchinskiĭ (30)]. The serum proteins of rat embryos differ immunologically from those of adults and are gradually replaced by adult types with increasing age. The rate of such replacement differs in the different fractions of serum proteins [Gurvich & Karsaevskaya (31)]. The serum proteins of both adults and embryos are formed in the liver, but they do not cross the placenta [Shmerling (32)]. In the development of hen's eggs there is no dynamic exchange between the amino acids of the proteins of white, yolk, and embryo [Orekhovich *et al.* (33)]. In postembryonic life, the tissue proteins of young animals exchange amino acids faster than do those of adults [Toropova (34)], but the rate of renewal of plasma, liver, spleen, and lung proteins is greater in older animals than in young. Kidney protein is an exception to this statement [Salganik (35)]. Glutamine and asparagine, which are important substances widely distributed in the tissues, [Ferdman & Silakova (36); Lestrovnaya (37); Olenicheva (38)] appear early in developing embryos. Glutamine appears first [Shmerling & Mogilevskaya (39)].

There have been many studies on the metabolism of specific proteins in various organs and tissues. Plasma proteins are regenerated rapidly after bleeding, although the immediate rise in the amount of such proteins in blood may be caused by nervously controlled discharge of stored proteins before synthesis increases them [Rodionov *et al.* (40)]. Kurokhtina (41) claims that different fractions of serum proteins are regenerated at the same rate. Aldehydes and certain quinones added to the blood increase the concentration of the α - and β -globulin fractions, probably by forming adsorption complexes. This may be a mechanism for the transport of these compounds [Troitskiĭ & Sorkina (42)]. Citral [Troitskaya (43)] and dextran [Melik-Sarkisyan & Rozenfel'd (44)] also form complexes with plasma proteins.

After partial hepatectomy there is rapid regeneration of liver protein, which occurs chiefly in special cytoplasmic granules, distinct from the mitochondria. The specific function of these granules is protein synthesis [Khesin (45); Khesin & Petrashkaite (46)]. Similar cytoplasmic granules which synthesize protein are found in the pancreas [Khesin *et al.* (47)] and kidney [Titova & Shapot (48)]. Enhanced functional loading of the liver stimulates liver regeneration [Solopaev (49)]. Regeneration in the cell nuclei is slower at first than in tissues as a whole [Perevoshchikova (50)]. The major source of energy is ATP [Livanova (51)]. The effect of biotin deficiency in reducing protein synthesis is not direct but results from its interference with the citrate cycle, which is also required for protein synthesis [Poznanskaya (52)].

The regeneration of skin proteins is a function of metabolic rate [Kholmulo (53)] and requires the presence of vitamin C. The various procollagens first formed may be considered successive stages of collagen formation in the different layers of the skin [Orekhovich & Pavlikhina (54)]. The protein of spermatozoa is localized in the tails where ATP is found [Engel'gardt & Burnasheva (55)].

Various pathological conditions are accompanied by changes in protein metabolism. There is a large literature on protein metabolism in tumor cells.

In general, such cells synthesize protein more slowly than do normal cells [Gavrilova (56); Zbarskiĭ & Perevoshchikova (57)]. Pentylenetetrazole (Metrazol; Cardiazol) and electric shock also reduce protein synthesis in all tissues [Rozengart & Maslova (58)].

Diets severely deficient in protein produce a number of effects on the organism. There are relatively gross effects, such as decrease in ovarian function [Ryabinina (59)] or in the content of solid matter of intestinal juice [Gadzhieva (60)]. Enzymatic effects include decrease in xanthine oxidase activity of the liver [Polezhaeva-Shifman (61)], of carbonic anhydrase activity in blood, muscle, and gastric mucosa [Merezhinskiĭ & Gutovskaya (62)], and of transaminase in the liver [Berezovskaya & Smirnova (63)]. Metabolic reactions of alanine [Kaplanskiĭ & Azyavchik (64)], tyrosine [Kazantseva & Kaplanskiĭ (65)], and tryptophan [Velikodvorskaya (66)] are slowed down. Production of various proteins is decreased. These include antibodies and phagocytes of the blood [Moroz (67)], phosphorus compounds of bone marrow [Kaplanskiĭ *et al.* (68)], and liver proteins [Kaplanskiĭ & Kuzovleva (69)]. Regeneration of muscle protein is greatly slowed [Kagan (70)], and the severity of rachitic symptoms is increased [Perkovich (71)]. Deficiencies of the specific amino acids methionine and lysine produce histological changes in various organs [Shosh (72)] and reduce conditioned reflex activity, indicating specific effects on the higher nervous centers. Lysine deficiency has the most serious effects in this respect [Veis *et al.* (73)].

In addition to the general studies of protein metabolism, there have been many investigations on the metabolism of nucleoproteins and nucleic acids. The particular importance of DNA for heredity has been shown by Spirin *et al.* (74), who demonstrate that when bacteria undergo a variation which results in changes in morphological, immunological, and biochemical properties, the composition of DNA in the cell nuclei changes, but the composition of RNA in the cytoplasm remains almost constant. Differences between DNA and RNA occur in vitamin C-deficient animals. The uptake of P^{32} by the DNA of intestinal mucosa drops, while that of RNA does not change [Gol'dshtein *et al.* (75); Gol'dshtein (76)]. The rate of P^{32} uptake varies with the firmness of binding of the nucleic acid to protein, indicating a different function for the firmly and loosely bound nucleic acids [Manoilov & Orlov (77); Demidova (78)]. Only the fraction of RNA which is firmly bound to the protein undergoes oxidative phosphorylation [Pavlova (79)]. Mitotic activity of cells is closely connected with the presence of nucleic acids [Ermolenko (80)].

The effects of radiation on protein metabolism have been extensively investigated. In radiation sickness the motor function of the gastrointestinal tract is decreased and absorption of proteins as measured with radioactive methionine falls. The entire uptake of methionine by all tissues is thus decreased [Okulov (81)]. Lethal doses of x-rays cause a negative nitrogen balance [Fedorova (82)]. Acute or chronic exposure of rabbits to ionizing radiation causes an increase in the globulin fraction of serum proteins and a decrease in albumin. If the rabbits survive, their protein metabolism is

disturbed for months [Fastyuchenko & Varshavskii (83)]. The antigenic properties of serum proteins are changed [Zil'ber *et al.* (84)].

Protein metabolism of specific organs is greatly changed. Muscular tissue is relatively insensitive to radiation damage, being affected only at very high doses and in the later stages [Tuzhilkova (85)], although the excretion of creatine is increased at lower levels of irradiation [Fedorova & Larina (86)]. Bone marrow loses its ability to take up P^{32} to form phospholipides, nucleic acids, and, to a lesser extent, phosphoproteins [Turovskii (87)]. The free amino acid content of bone marrow also drops [Kasavina & Spektor (88)]. Uptake of phosphorus by proteins of liver, kidney, spleen, and small intestine drops after irradiation. This effect is particularly noticeable in the nucleic acid fraction in spleen and small intestine. It differs somewhat in liver and kidney. There is probably a difference in behavior of stable and labile phosphorus in the DNA, which results in a difference in behavior of different organs [Orlov (89)]. The major change in the liver is in the RNA and in the lipoproteins [Il'ina (90); Il'ina *et al.* (91)]. There is considerable disturbance in purine biosynthesis [Kritskii (92)]. The spleen is very sensitive to the action of ionizing radiation [Kuzin & Budilova (93)]. Both DNA and RNA content are decreased, and during recovery the RNA content rises sharply even before tissue regeneration begins. This suggests that RNA plays a part in the recovery process [Luchnik (94)]. Resumption of DNA formation after irradiation is more difficult in the spleen than in the kidney [Manollov *et al.* (95)]. The sensitivity of the sulfhydryl groups of some enzyme systems to poisons is increased by radiation [Gintsburg *et al.* (96)].

Carbohydrate metabolism.—The energy for most cellular reactions, including amino acid synthesis and renewal of proteins, depends largely on the oxidation of carbohydrates. The activity of adenosine triphosphatase is a measure of cell and tissue regeneration [Braunshein & Azarkh (97); Yakovleva (98)]. There is some evidence that phosphate groups in RNA have high-energy properties resembling those of ATP [Bresler & Rubina (99)].

The relationship of the various stages of carbohydrate metabolism to one another changes as the animal develops. In embryonic muscle tissue, oxidative processes are very intense, accompanied by oxygen absorption, greater binding of inorganic phosphate, and formation of fructose diphosphate in the aerobic stage. Fluoride ion has little effect on this stage [Vul'fson (100)]. Phosphorus exchanges freely across the placenta, and maternal phosphorus metabolism is increased [Lessakova (101)]. After birth, oxidative phosphorylation of the usual type becomes characteristic. Glucose carbon goes into the formation of complex polysaccharides and glycoproteins at all times [Kuzin *et al.* (102)], as well as forming glycogen. Glycogen differs somewhat in chain length in various species [Stepanenko & Afanas'eva (103); Afanas'eva & Stepanenko (104)]. Most of the glycogen in muscle fibers is bound to the anisotropic substance of the myofibril, though a small amount is found in the sarcoplasm [Shubich (105)].

A number of studies have shown nervous control of muscle metabolism.

Denervation of the muscle produces an immediate decrease in ATP in the muscle and a general lowering of glycolytic activity [Kanashenok (106)]; Epel'baum & Kantor (107)]. Addition of ATP to the muscle reduces the dystrophy which results from denervation [Ferdman (108)]. A number of the enzymes involved in carbohydrate metabolism are damaged, including most of those concerned with various phosphorylations [Sheves (109); Telepneva (110); Slozhenikina (111)]. The effects of tenotomy are similar, but less pronounced (107, 109, 110). Decerebration also alters carbohydrate metabolism in muscle [Kanashenok (112); Tsinkalov's'ka (113)], raises blood sugar [Kakhana & Telenkevich (114)], and increases the creatine content of muscle [Kanashenok (115)].

The role of carnosine and anserine in muscle metabolism has been much studied. Carnosine formation precedes that of anserine in chicken embryos [Skvortsova (116)]. The site of carnosine and anserine formation is in the muscle [Razina (117)]. The compounds appear to increase oxidative phosphorylation and formation of ATP [Meshkova & Zaitseva (118); Nagradova (119)]. The action may be on an intermediate stage in ATP formation [Severin & Meshkova (120)]. The compounds are also effective in smooth muscle [Dikanova (121)].

Rapid anaerobic and aerobic glycolysis occur in leucocytes [Chernyak (122)]. The aerobic metabolism (production of lactic acid under aerobic conditions) is similar to that of thrombocytes [Luganova *et al.* (123)] and cancer cells. Thus, this type of metabolism is not specific for malignancies [Luganova *et al.* (124)]. In human erythrocytes, glycolysis is the only source of energy, though in rabbit erythrocytes aerobic oxidation is about as important [Vladimirov *et al.* (125)]. The adenosine triphosphatase activity of nucleated erythrocytes in pigeons and rabbits is concentrated on the surface, leading to the suggestion that this is a specific enzyme. It has been called ectoadenosine triphosphatase by Venkstern & Engel'gardt (126). In anemia there is injury to carbohydrate metabolism which is probably chiefly caused by hypoxia [Chirkov (127)].

The alteration in blood sugar level produced by nicotinic acid is not the result of mediation through insulin or epinephrine [Khaes (128)] but results from nerve reflexes whose nature depends on dose of nicotinic acid and physiological state of the nervous system [Khaes (129)].

Alloxan diabetes is a tool which has been much used to study carbohydrate metabolism. It has been shown that uptake of glucose by the liver is suppressed as a result of decreased permeability of the liver cells in this condition [Petrova (130)]. Phosphorylation in the liver cells also decreases [Petrova & Bekina (131)]. The synthesizing action of phosphorylases is increased, but phosphoglucomutase activity is diminished [Petrova (132)], and the amount of fructose present rises [Bekina & Petrova (133)]. In muscle, the splitting of glycogen is decreased in alloxan diabetes in rabbits [Petrova (134)]. Formation of glycogen from glucose under the influence of epinephrine is suppressed [Stepanenko *et al.* (135)].

Pharmacological stimulation of the central nervous system increases

blood sugar and the severity of alloxan diabetes [Smirnov (136)]. Suppression of such stimuli prevents development of diabetic hyperglycemia [Veller & Charnaya (137)]. The central nervous system also controls the response of both normal and diabetic dogs to repeated glucose injections [Leites *et al.* (138)].

The effects of hypnotics on carbohydrate metabolism in the organism are somewhat variable [Idel'chik (139); Il'in & Titova (140); Pravotorova (141)] probably because different drugs affect different enzyme systems [Tseitlin (142)]. The most important factor which is affected by hypnotics, however, is the control by the central nervous system [Gruzdeva (143); Karyakina (144)].

Such central nervous system control is further indicated by investigations with conditioned reflexes. A reflex can be established by irritation of receptors in the mouth during eating [Kanfor (145)] which affects intestinal absorption of carbohydrates [Bannikova (146)] and distribution of sugar in the organism (145). Variations in the ratio of fat to carbohydrate in the diet may strengthen or weaken the reflex [Sergeeva (147)].

Total irradiation of dogs with resulting production of radiation sickness causes disturbance in liver function [Graevskaya & Keilina (148); Blokhin *et al.* (149)]. The decrease in liver glycogen is to some extent caused by failure of intestinal tract function with resulting reduction in glucose for the tissues [Tropova (150)], but there is also specific suppression of the phosphorylation of glycogen in the liver [Keilina (151)]. Adenosine triphosphatase is also affected [Prokudina (152)]. β -Irradiation of exteriorized small intestine by Sr^{90} causes a 20 to 30 per cent rise in blood sugar. Denervation of the intestine before irradiation prevents this phenomenon, indicating it is of a reflex nature [Keilina & Komarov (153)].

Lipide metabolism.—The number of studies of lipide metabolism is much smaller than that of protein or carbohydrate metabolism studies. Much of the attention in this field has centered on the problem of the mechanism of atherosclerosis and the combination of cholesterol with proteins, especially in the globulin fraction of blood serum [Bavina & Kritsman (154); Tarasova (155)]. The rate of conversion of these serum proteins into the proteins of various organs and tissues is decreased in atherosclerosis, in which disease sterol-protein complexes exist [Kritsman & Bavina (156); Kritsman *et al.* (157)]. The number of free sulfhydryl groups in these proteins is increased, indicating differences in their functional groups [Alekseeva (158)]. Probably the individual enzyme systems are changed [Bavina & Alekseeva (159)].

The nature of the protein-steroid complexes is also changed under the influence of deficiency or excess of vitamin D, as indicated by differences in phosphorus content in their fractions [Areshkina *et al.* (160)]. Vitamin-A deficiency results in decreased oxidation of fatty acids and a consequent increase in the amounts of unsaturated fatty acids in the liver, kidneys, and lungs [Leutskii & Lyubovich (161)].

Miscellaneous metabolic studies.—Iron is assimilated and utilized better

in the presence of dietary phosphoprotein such as casein [Kruchakova (162)]. In healthy animals much dietary iron is stored in the liver rather than being used for hemoglobin synthesis. In anemia, on the other hand, most dietary iron is used in such synthesis [Kurdyballo (163)]. Radiation sickness prevents hemoglobin synthesis and causes continued accumulation of ingested iron in the liver [Shepshelevich (164)]. Neuroses resulting from conflicts in conditioned or unconditioned reflexes may cause decreased hemoglobin formation [Fokina (165)].

The specific dynamic action (SDA) of foods may result from conditioned reflexes. Continued feeding of fats leads to a conditioned increase in the SDA which they cause [Makarova (166)]. Changes in the SDA of foods after decortication in dogs indicate that the cortex participates in both conditioned and unconditioned reflex mechanisms for SDA [Zhmakin (167)]. Seasonal variations in SDA in wild animals, which are not observed in laboratory rats, are probably also caused by reflex mechanisms [Isaakyan (168)]. Vitamin-B₂ content of the body is regulated by nervous impulses both directly and by way of the liver, as is shown by the changes in riboflavin content when various organs are denervated [Danetskaya (169)].

Metabolism of the brain.—The great interest among Soviet physiologists in the control of bodily functions by the central nervous system has led to a large number of studies on the metabolism of the brain and the various factors that affect it.

During ontogenesis, the uptake of P³² by all brain tissues is high in the early stages of embryonic development. It falls at the time of birth and then rises again. Incorporation of phosphorus into RNA is always higher than into DNA, and uptake by RNA continues for a much longer time [Skvirskaya & Chepinoga (170)]. The renewal rate of phosphorus in phospholipides is rapid in young animals and lower in adults [Manukyan (171)]. The cessation in increase in amount of brain nucleic acids occurs in different species at times which are determined by development of full function in the enzyme systems [Manukyan (172)].

The activity of cytochrome oxidases in the brain varies from species to species, being lowest in cold-blooded animals and highest in warm-blooded land animals [Verzhbinskaya (173)]. Phosphoproteins are a distinct fraction of brain tissue and undergo oxidative phosphorylation [Lisovskaya (174)]. Glycine containing C¹⁴ is taken up by the brain, and the radioactive carbon is found in serine, cystine, and C₂ amino acids [Sadikova & Skvortsevich (175)]. Phosphoserine is a major component of brain phosphoproteins [Vladimirov *et al.* (176)]. Radioactive phosphorus is taken up most rapidly when it is injected directly into the brain. Injected phosphorus remains longer in the brain than in other organs. Exchange of phosphorus in the phospholipides is especially slow [Grodzenskiĭ & Avakyan (177)]. Proteins which contain sulfhydryl groups are widely distributed in different brain tissues and are probably concerned in cerebral function [Savich & Yakovlev (178); Panchenko (179)]. They are higher in human brain tissue than in other

organs and tissues. This may be related to energy production in the brain [Dmitriev (180)]. Proteins of nerves are metabolized at a lower rate than those of the brain [Palladin *et al.* (181)].

As in the case of growing animals, renewal of adult RNA continues in all brain tissues when DNA renewal has stopped [Khor'kova (182)]. The distribution of nucleic acids differs in different parts of the brain but correlations with functional activity have not been clearly established [Skvirskaya & Silich (183)]. X-ray irradiation of the brain does not alter the RNA content greatly but does change the DNA content. The amount of certain phosphorus compounds of unknown composition which are associated with the nucleic acids is also decreased [Golubtsova *et al.* (184)].

The energy for phosphorylative syntheses appears to depend on ATP hydrolysis in the brain [Engel'gardt & Lisovskaya (185)]. Excitation of the central nervous system by electric shock or by drugs such as amphetamine causes increased renewal rate of proteins [Nechaeva (186); Palladin *et al.* (187)]. Narcosis has a somewhat depressant effect [Vladimirov & Urinson (188)]. Hypoxia increases the renewal rate of proteins [Smirnov & Chetverikov (189)], while exposure to high oxygen pressures which cause convulsions inactivates glutamine synthesis and increases free glutamic acid and ammonia [Gershenovich & Krichevskaya (190)]. The content of ammonia in the brain may be taken as a measure of its protein metabolism [Vladimirova (191)].

The labile phosphorus content of the brain is largely centered in inorganic phosphate, ATP, and creatine phosphate, and is thus a measure of the carbohydrate metabolism and energy production in the brain. The content of labile phosphorus varies in different parts of the brain [Baranov (192)], but increases with growth of the individual [Smirnov & Chetverikov (193)] and is higher in the brains of higher animals [Verzhbinskaya & Volkova (194)]. In cold-blooded animals the temperature determines the activity [Volkova (195); Savchenko (196)]. Carbohydrate metabolism is closely connected with the functional state of the brain. Suppression of irritability of the cerebral cortex by injection of monobromoacetate results from suppression of glycolysis and failure of ATP resynthesis. ATP injection temporarily reverses this process [Malkiman (197)].

Suppression of brain function by natural or narcotic sleep somewhat lowers phosphate metabolism [Smirnov (198)]. In hypoxia induced by sodium nitrite injection, the glycogen content of the brain decreases [Prokhorova *et al.* (199)]. In "clinical death" from severe blood loss, all synthetic processes in the brain stop, but during resuscitation they revive again. Resynthesis of ATP and creatine phosphate in the brain is not the sole condition for revival, however [Shuster (200); Petrov *et al.* (201)]. During muscular exercise, hydrolysis of ATP and creatine phosphate increases [Yakovlev (202)]. Administration of oxygen under pressure, leading to convulsions, does not change the level of brain ATP in guinea pigs [Gershenovich & Bronovitskaya (203)]. Glutamic acid stimulates ATP synthesis [Ivanov & Rozenberg (204)]. Stimulating drugs produce varied effects. Methamphetamine (Pervitin) in-

creases work capacity and stimulates the central nervous system. It increases the phosphate activity of ATP and somewhat decreases glycogen in the brain [Palladin & Rybina (205); Khaikina *et al.* (206)]. Pentylenetetrazole (Cardiazole), which stimulates the cortex but does not increase work capacity, decreases phosphate activity in ATP (205). Stimulation by caffeine or by conditioned reflexes increases rate of conversion of ATP to ADP [Vladimirova (207); Sytinskii (208)].

Total irradiation of rabbits causes a marked drop in glycolytic activity of the brain, corresponding to a depressed functional state of the central nervous system [Maev's'ka (209)]. In later stages of irradiation there is an actual outflow of glucose from the brain (148).

About half the lipid fraction of the brain is bound to protein. More of the lipides and cholesterol are found in the white matter than in the gray [Nedzvetskii & Ratnitskaya (210); Polyakova (211)]. In vitamin-A deficiency, brain cholesterol decreases [Leutskii (212)]. Although the metabolism of phospholipides tends to be slower than that of the phosphoproteins, there is a phospholipide fraction with more rapid phosphorus uptake, which appears to be related to the functional state of the brain, decreasing in sleep [Vladimirov *et al.* (213)]. The brain is capable of synthesizing coenzyme A, an ability which is decreased by x-ray irradiation [Trufanov & Popova (214)]. In light sleep the brain, unlike most other tissues, takes up less vitamin B₁ than during wakefulness, though in heavy sleep the uptake is decreased in almost all tissues [Savitskii (215)]. All these facts combine to indicate that the functional state of the brain is reflected in its metabolic state.

ENDOCRINOLOGY

Introduction.—Much Soviet endocrinology is of a clinical nature. This is particularly true of disorders of the thyroid gland, which seem to present a serious medical problem in the Soviet Union. However, there has recently been greater interest in the more strictly physiological side of endocrinology. With the establishment in 1955 of the journal *Problems of Endocrinology and Hormone Therapy*, the number of papers in the field has increased considerably. The adrenal and thyroid glands have been the subject of the largest number of papers, probably because of their rather close relationship to the nervous system. It is the interrelationship between the nervous and endocrine systems that attracts many Soviet investigators, though the varied effects of the various hormones on different bodily processes and different types of metabolism have by no means been neglected.

Thyroid gland.—Uptake of iodine by the thyroid gland begins in the embryo as soon as the gland differentiates [Matskevich & Mamul (216)]. The uptake is greatest when the gland is functioning at the highest level [Kasheenko (217)]. Vitamin C and members of the B complex improve iodine uptake by the thyroid [Siver *et al.* (218)].

The thyroid plays an important part in the renewal of proteins. When its activity is suppressed by 6-methylthiouracil, there is an increased rate of protein breakdown and a decrease in rate of synthesis [Epel'baum &

Dergorisova (219)]. The failure of regeneration in muscular tissue which results from such suppression of activity may lead to muscular dystrophy in mice [Kovalenko (220)]. Thyroxin increases formation of amino acid oxidase in the liver when this function is depressed by a low protein diet. The increased activity is related to the sulfhydryl groups of the enzyme [Azyavchik (221)]. In hyperthyroidism there is a drop in sulfhydryl groups of this enzyme, probably caused by their oxidation [Gol'dshtein & Gotovtseva (222)].

The effects of thyroxin on a number of tissues have been noted. The breakdown of glycogen in the brain is increased, the amylase activity rises as does the process of phosphorylation, and the content of pyruvic and lactic acid drops [Potop (223)]. Liver protein metabolism is affected early in thyrotoxicosis, with a somewhat later disturbance in carbohydrate metabolism. The detoxification processes in the liver are also hindered [Mandl (224); Alekperov (225)]. Mitosis is increased in normal animals, and the increase produced in rats by extra illumination is mediated through the thyroid, since administration of methylthiouracil prevents this effect [Epova (226)]. Cancer cells do not show increased mitosis under the influence of thyroxin [Alov (227)]. The hormone stimulates the activity of the macrophage system [Voitkevich *et al.* (228)] and increases the excretion of excess water [Genes & Lesnoi (229)]. The sensitivity to shock from intense sound waves is also increased [Krushinskiĭ & Dobrokhtova (230)].

Thyroxin affects the action of other hormones. It permits more rapid establishment of insulin hypoglycemia [Rodkina (231)], changes the ratio of reduced and oxidized epinephrinlike substances in muscle in a complex fashion [Utevskii & Butom (232)], and enhances the effect of testosterone on secondary sex characteristics in the male [Vunder (233)].

There are strong reciprocal relations between the central nervous system and the thyroid gland. Even in the embryonic stages of development of this gland, drugs which depress the central nervous system reduce uptake of iodine, while stimulants such as caffeine and amphetamine increase it. This leads to the conclusion that the central nervous system controls the gland during its development [Mitskevich (234)]. Similar effects of drugs are found in adult animals [Kolli (235)]. The uptake of iodine is a specific function of the thyroid, but the structure of the gland is controlled by the brain, and any change in the work of the organ is signalled to the central nervous system. There is thus a close connection in function of thyroid and brain [Aleshin & Demidenko (236); Tarakanov (237)]. Emotional states and neuroses in animals affect both uptake of iodine and discharge of thyroxin into the blood [Amiragova (238); Arkhipenko (239)]. The whole state of the organism, including an active nervous system, is essential for proper thyroid function [Voitkevich (240); Vunder (241); Skebel'skaya (242)]. It is possible to establish conditioned reflexes which increase the blood pressure through alteration of thyroid function [Isichenko (243); Amiragova (244)], though there is evidence that the effects of the central nervous system may be transmitted to the thyroid through the pituitary or adrenals [Amiragova (245)].

X-ray irradiation of the brain reduces the symptoms of thyrotoxicosis [Gincherman & Ioffe (246)].

On the other hand, thyroxin affects the central nervous system. Thyroidectomy and methylthiouracil produce diffuse histological changes in nerve cells [Rabkina (247)]. Doses of thyroxin too small to affect the basal metabolism rate can produce changes in the brain cortex, especially in sensitive individuals [Baranov *et al.* (248)]. Higher nervous function is disturbed in thyrotoxicosis [Vasil'eva (249)]. Thyroxin can alter the central nervous system regulation of gastric evacuation [Genes & Lesnoi (250)] and of respiratory metabolism [Dzogoeva (251)].

Adrenal medulla.—Changes in the central nervous system have, of course, a pronounced effect on epinephrine secretion. Stimulation or depression of the activity of the brain is reflected not only in epinephrine content in the adrenals [Utevskii & Butom (252); Ozerova (253)], but also in other tissues, including the brain itself [Gershenovich *et al.* (254)]. Metabolic reaction products from epinephrine and especially norepinephrine are found not only in the adrenals [Emel'yanova (255)] but also in other organs such as the spleen, skeletal muscle, brain, liver, and heart [Osinskaya (256)]. In the heart, norepinephrine is bound to protein [Barts (257)]. When deoxycorticosterone is injected into the heart muscle, there is less decomposition of this complex than in control animals [Barts (258)]. Conditioned reflexes can be established which lead to epinephrine secretion [Poskalenko (259); Sveshnikova (260); Karagezyan (261)]. In alimentary hypercholesterinemia, there is an initial decrease in epinephrine content of the adrenals, but this gradually returns to normal [Pashchenko (262)]. Epinephrine causes a secondary rise in blood pressure after stimulation of the proximal end of the sciatic nerve, in dogs and cats. This is a result of discharge of vasopressin and is mediated through the central nervous system [Il'ina & Tonkikh (263)].

Adrenal cortex.—As with the other hormones, Russian endocrinologists believe that central nervous system stimuli, mediated through the pituitary, affect the production of corticoids [Mikhaïlova (264); Trachik (265)]. Drugs which affect the brain alter ACTH production [Anichkov (266)]. Conditioned reflexes can be established for its control or function [Mikhailova (267); Isichenko (268); Pyshina (269); Nikolov (270)]. Many of the effects of the cortical hormones are considered to be a result of their action on functional changes in the central nervous system brought about by their effects on metabolism in the nerve cells [Stroganova (271)].

Eskin (272) believes, on the basis of such evidence, that corticoid concentration in the blood does not control liberation of ACTH in response to stress. Rather, it is the action of the brain cortex on the hypothalamus which liberates the substance causing the pituitary to secrete ACTH. The brain, in turn, is responding to stimuli from the peripheral nerves. Newborn rats or mice secrete cortical hormones immediately, though in slightly different ratios from adults [Yudaev & Druzhinina (273)]. Their pituitary glands also form ACTH but do not liberate it into the blood in response to stress as in the adult. Eskin believes this is because the nervous pathways which

transmit the stress stimulus to the brain have not yet developed. Response to different forms of stress develops at different periods of growth, as the different parts of the nervous system are completed. The earliest response of the pituitary is to norepinephrine, which implies that the hypothalamic-hypophysiotropic factor of Guillemin & Rosenberg (274) is a norepinephrine-like substance.

The widespread effects of cortical hormones on many processes and tissues have been noted. Although ACTH and cortisone have almost no effect on intensity of metabolism of serum proteins [Mednik (275)], cortisone and deoxycorticosterone increase incorporation of radioactive glycine into liver protein [Yudaev *et al.* (276)] and increase the activity of deaminases and tyrosine oxidase, and urea formation in the liver [Protasova (277)]. Tissue respiration is increased by small doses of ACTH and reduced by large ones [Seifulla (278)]. A number of studies relate to the reduction of inflammatory processes by corticoids [Smirnov (279); Serdyukova & Yufina (280); Mednik (281); Yudaev *et al.* (282)].

ACTH decreases the ability of the thyroid gland to take up iodine even when the adrenals have been removed [Skebel'skaya (283)]. It suppresses growth of the ovaries and uterus and with prolonged use decreases the action of the adrenals and thyroid [Eskin (284)]. ACTH increases the hyperglycemia of diabetes [Leites & Yakusheva (285); Rabkina (286); Veller & Charnaya (287)].

Exposure to ionizing radiation decreases the activity of the adrenal cortex and lowers the amount of vitamin C which it contains. These reactions are reflections of total response of the organism to radiation sickness rather than the result of specific action on the adrenals, since they do not occur when only the adrenals are irradiated [Tret'yakova (288); Bozhenko (289); Zhorno (290)]. The mineralocorticoids appear to be most seriously affected [Pavlova & Rabkina (291)]. Species differences in sensitivity to irradiation occur [Orlova *et al.* (292)].

Insulin.—Most of the studies on insulin are either strictly clinical, and so beyond the scope of this paper, or have already been discussed under the various phases of carbohydrate metabolism. However, there are a number of studies on the relation of the pancreatic hormone to the central nervous system which should be mentioned. Insulin increases the sensitivity of the central nervous system independently of its hypoglycemic action [Katorovich (293)]. Insulin deficiency suppresses conditioned reflex activity [Mityushov (294)] and damages the central nervous system and peripheral nerves [Veller *et al.* (295)]. The functional state of the higher levels of the nervous system in turn regulates and coordinates insulin production [Yankelevich (296)].

Sex hormones.—Androgens stimulate the activity of arginase and decrease the activity of transaminase, especially in males [Shorm & Shveitsar (297)]. Phosphorus metabolism is affected by castration, but the effects are variable in different organs [Makarevich-Gal'perin & Ushenko (298)]. Structural changes, as indicated by polarographic studies, are found in the blood pro-

teins of castrated animals [Novikova & Kuz'menko (299)]. Castration of either males or females slows evacuation of the stomach. This effect may be mediated through the central nervous system [Genes (300); Plavskaya (301)]. Estrone stimulates mitotic activity in the uterus but not in other organs [Alov (302)].

Castration or administration of progesterone or estrogens alters the character of brain waves in an electroencephalogram. Progesterone slows the waves; estrogen increases their frequency [Khrustaleva (303)]. Conditioned reflexes may be altered in animals by castration or by administration of sex hormones [Isichenko (304); Vartapetov *et al.* (305); Fel'berbaum (306)], though the hormones have no effect on conditioned reflexes in older human beings with increasing age or at the climacteric [Samtsova (307); Blagosklonnaya (308)].

Irradiation of the gonads of frogs with x-rays reduces their response to gonadotropic hormones. The loss in responsiveness comes on gradually after the exposure [Kashchenko & Pushnitsyna (309)]. The ovaries are less sensitive to irradiation during functional rest than during activity. In male frogs, severe irradiation leads to degeneration of the reproductive organs, accompanied by vigorous secretion of gonadotropic hormones [Kashchenko & Pushnitsyna (310)].

Miscellaneous studies on hormones.—The production of other hormones is also affected by the central nervous system. Hypocalcemia stimulates production of parathyroid hormone by acting on the central nervous system [Benetato *et al.* (311)]. Mobilization of the antidiuretic hormone can be influenced by conditioned reflexes [Kharvat & Golechek (312); Kakhana (313)]. Sympathetic impulses stimulate prolactin formation [Ryabushko (314)].

LITERATURE CITED

1. Genes, S. G., *Uspekhi Sovremennoi Biol.*, **35**, 229-56 (1953)
2. Aleshin, B. V., *Uspekhi Sovremennoi Biol.*, **39**, 276-98 (1955)
3. Speranskaya, E. N., *Fiziol. Zhur. S.S.S.R.*, **42**, 418-24 (1956)
4. Stekol, J. A., *Ann. Rev. Biochem.*, **26**, 611-44 (1958)
5. Shishova, O. A., *Biokhimiya*, **21**, 111-18 (1956)
6. Braunshtein, A. E., and Azarkh, R. M., *Biokhimiya*, **22**, 430-38 (1957)
7. Efimochkina, E. F., Ottesen, B. V., Alekseev, I. V., and Bichin, L. P., *Voprosy Med. Khim.*, **3**, 440-50 (1957)
8. Goryachenkova, E. V., *Doklady Akad. Nauk S.S.S.R.*, **93**, 319-20 (1953)
9. Volovnik, B. Ya., *Biokhimiya*, **20**, 490-94 (1955)
10. Balakhovskii, S. D., and Drozdova, N. N., *Biokhimiya*, **22**, 330-35 (1957)
11. Balakhovskii, S. D., and Kuznetsova, I. V., *Doklady Akad. Nauk S.S.S.R.*, **118**, 331-33 (1958)
12. Cherkes, L. A., Fil'chagin, N. M., and Dinerman, A. A., *Biokhimiya*, **20**, 140-45 (1955)
13. Dinerman, A. A., *Voprosy Pitaniya*, **16**(1), 36-43 (1957)
14. Cherkes, L. A., and Fil'chagin, N. M., *Biokhimiya*, **21**, 64-70 (1956)
15. Plyshevskaya, E. G., and Rozenfel'd, E. L., *Doklady Akad. Nauk S.S.S.R.*, **94**, 1141-44 (1954)
16. Yushkevich, N. L., and Kedrovskii, B. V., *Biokhimiya*, **21**, 422-28 (1956)
17. Kedrovskii, B. V., and Yushkevich, N. L., *Biokhimiya*, **22**, 1023-27 (1957)
18. Konikova, A. S., Kritsman, M. G., and Samarina, O. P., *Biokhimiya*, **19**, 440-48 (1954)
19. Samarina, O. P., Kritsman, M. G., and Konikova, A. S., *Biokhimiya*, **21**, 10-16 (1956)
20. Kritsman, M. G., Sukhareva, B. S., Samarina, O. P., and Konikova, A. S., *Biokhimiya*, **22**, 449-59 (1957)
21. Orekhovich, V. N., Kurokhtina, T. P., and Buyanova, N. D., *Biokhimiya*, **18**, 706-8 (1953)
22. Medvedev, Zh. A., *Biokhimiya*, **21**, 627-32 (1956)
23. Savitskii, I. V., and Leshchinskii, A. F., *Med. Radiol.*, **1**(6), 82-90 (1956)
24. Kazanova, I. L., and Arekhovich, V. N., *Doklady Akad. Nauk S.S.S.R.*, **93**, 875-78 (1953)
25. Kuzovleva, O. B., *Biokhimiya*, **19**, 453-60 (1954)
26. Uchitel, I. Ya., and Konikova, A. S., *Biull. eksptl. biol. med.*, **44**(7), 85-89 (1957)
27. Zamanskii, L. N., Lopushanskii, A. I., and Siver, P. Ya., *Doklady Akad. Nauk S.S.S.R.*, **99**, 177-79 (1954)
28. Kasavina, B. S., *Trudy 2-oi Nauch. Konf. po Vozrastnoi Morfol. i Fiziol.*, 194-204 (Moscow, 1955)
29. Ivanov, I. I., Yur'ev, V. A., Kadykov, V. V., Krymskaya, B. M., Moiseeva, V. P., and Tukachinskii, S. E., *Biokhimiya*, **21**, 591-95 (1956)
30. Kasavina, B. S., and Torchinskii, Yu. M., *Biokhimiya*, **21**, 510-15 (1956)
31. Gurvich, A. E., and Karsaevskaya, N. G., *Biokhimiya*, **21**, 746-54 (1956)
32. Shmerling, Zh. G., *Biokhimiya*, **19**, 683-87 (1954)
33. Orekhovich, V. N., Leviant, M. I., and Levchuk-Kurokhtina, T. P., *Biokhimiya*, **19**, 610-15 (1954)
34. Toropova, G. P., *Voprosy Pitaniya*, **14**(4), 12-14 (1955)

35. Salganik, R. I., *Biokhimiya*, **19**, 641-44 (1954)
36. Ferdman, D. L., and Silakova, A. I., *Doklady Akad. Nauk S.S.S.R.*, **92**, 1011-14 (1953)
37. Lestrovnaia, N. N., *Biokhimiya*, **19**, 478-84 (1954)
38. Olenicheva, L. S., *Biokhimiya*, **20**, 165-72 (1955)
39. Shmerling, Zh. G., and Mogilevskaya, Z. G., *Biokhimiya*, **19**, 30-36 (1954)
40. Rodionov, V. M., Uspenskaya, V. D., and Zamyatkina, O. G., with Grunt, T. P., and Polyakova, V. P., *Voprosy Med. Khim.*, **3**, 255-68 (1957)
41. Kurokhtina, T. P., *Biokhimiya*, **19**, 16-18 (1954)
42. Troitskiĭ, G. V., and Sorkina, D. A., *Biokhimiya*, **21**, 784-92 (1956)
43. Troitskaya, N. A., *Biokhimiya*, **18**, 151-58 (1953)
44. Melik-Sarkisyan, S. S., and Rozenfel'd, E. L., *Biokhimiya*, **22**, 730-35 (1957)
45. Khesin, R. B., *Biokhimiya*, **19**, 407-13 (1954)
46. Khesin, R. B., and Petrashkalte, S. K., *Biokhimiya*, **20**, 597-609 (1955)
47. Khesin, R. B., Petrashkalte, S. K., Tolyushis, L. E., and Paulauskalte, K. P., *Biokhimiya*, **22**, 501-15 (1957)
48. Titova, G. V., and Shapot, V. S., *Biokhimiya*, **20**, 485-89 (1955)
49. Solopaev, B. P., *Biull. eksptl. biol. med.*, **43**(5), 109-13 (1957)
50. Perevoshchikova, K. A., *Biull. eksptl. biol. med.*, **44**(9), 59-63 (1957)
51. Livanova, N. B., *Biokhimiya*, **22**, 578-86 (1957)
52. Poznanskaya, A. A., *Biokhimiya*, **22**, 668-76 (1957)
53. Khomul'ko, G. V., *Doklady Akad. Nauk S.S.S.R.*, **90**, 313-16 (1953)
54. Orekhovich, V. N., and Pavlikhina, L. V., *Voprosy Med. Khim.*, **3**, 195-201 (1957)
55. Engel'gardt, V. A., and Burnasheva, S. A., *Biokhimiya*, **22**, 554-60 (1957)
56. Gavrilova, K. I., *Biull. eksptl. biol. med.*, **39**(2), 36-39 (1955)
57. Zbarskiĭ, I. B., and Perevoshchikova, K. A., *Biokhimiya*, **22**, 295-304 (1957)
58. Rozengart, V. I., and Maslova, M. N., *Biokhimiya*, **22**, 946-53 (1957)
59. Ryabinina, Z. A., *Biull. eksptl. biol. med.*, **43**(4), 102-7; (5), 113-17 (1957)
60. Gadzhieva, Z. M., *Biull. eksptl. biol. med.*, **42**(9), 22-24 (1956)
61. Polezhaeva-Shifman, A. S., *Biokhimiya*, **20**, 57-65 (1955)
62. Merezhinskiĭ, M. F., and Gutovskaya, A. V., *Voprosy Pitaniya*, **16**(1), 65-69 (1957)
63. Berezhovskaya, N. N., and Smirnova, N. B., *Biokhimiya*, **21**, 457-60 (1956)
64. Kaplanskiĭ, S. Ya., and Azyavchik, A. V., *Biokhimiya*, **21**, 755-59 (1956)
65. Kazantseva, V. S., and Kaplanskiĭ, S. Ya., *Biokhimiya*, **21**, 528-33 (1956)
66. Velikodvorskaya, G. A., *Voprosy Med. Khim.*, **3**, 292-300 (1957)
67. Moroz, A. P., *Voprosy Pitaniya*, **16** (1), 43-48 (1957)
68. Kaplanskiĭ, S. Ya., Zamyatkina, O. G., and Khesin, R. B., *Biokhimiya*, **18**, 552-58 (1953)
69. Kaplanskiĭ, S. Ya., and Kuzovleva, O. B., *Biokhimiya*, **22**, 162-70 (1957)
70. Kagan, M. Yu., *Doklady Akad. Nauk S.S.S.R.*, **91**, 683-86 (1953)
71. Perkovich, E. A., *Pat. fiziol. Moskva*, **1**(4), 38-43 (1957)
72. Shosh, I., *Voprosy Pitaniya*, **14**(5), 16-20 (1955)
73. Veis, P., Shosh, I., Gati, T., Karmosh, P., and Rigo, Ya., *Voprosy Pitaniya*, **15** (1), 15-21 (1956)
74. Spirin, A. S., Belozerskiĭ, A. N., Kudlaĭ, D. G., Skavronskaya, A. G., and Mitereva, V. G., *Biokhimiya*, **23**, 154-63 (1958)
75. Gol'dshtein, B. I., Gerasimova, V. V., and Kondrat'eva, L. G., *Biokhimiya*, **19**, 531-42 (1954)

76. Gol'dshtein, B. I., *Vitaminy, Akad. Nauk Ukr.*, **2**, 123-33 (1956)
77. Manollov, S. E., and Orlov, A. S., *Biokhimiya*, **18**, 456-61 (1953)
78. Demidova, P. D., *Biokhimiya*, **23**, 47-51 (1958)
79. Pavlova, M. V., *Doklady Akad. Nauk S.S.S.R.*, **92**, 641-43 (1953)
80. Ermolenko, L. M., *Biull. eksptl. biol. med.*, **44**(12), 102-6 (1957)
81. Okulov, N. M., *Med. Radiol. Moskva*, **1**(5), 41-45 (1956)
82. Fedorova, T. A., *Trudy Vsesoyuz. Konf. Med. Radiol., Eksptl. Med. Radiol.*, 103-8 (1957)
83. Fastyuchenko, O. V., and Varshavskii, B. M., *Trudy Vsesoyuz. Konf. Med. Radiol. Eksptl. Med. Radiol.*, 121-22 (1957)
84. Zil'ber, L. A., Artamonova, V. A., Frank, G. M., and Snezhko, A. D., *Med. Radiol. Moskva*, **1**(2), 17-23 (1956)
85. Tuzhil'kova, T. N., *Med. Radiol. Moskva*, **1**(4), 14-21 (1956)
86. Fedorova, T. A., and Larina, M. A., *Med. Radiol. Moskva*, **1**(6), 36-40 (1956)
87. Turovskii, V. S., *Ezhegodnik Inst. Eksptl. Med., Akad. Med. Nauk S.S.S.R.*, 439-40 (Leningrad, 1955)
88. Kasavina, B. S., and Spektor, E. B., *Med. Radiol. Moskva*, **1**(3), 72-80 (1956)
89. Orlov, A. S., *Med. Radiol. Moskva*, **1**(2), 65-70 (1956)
90. Il'ina, L. I., *Biull. eksptl. biol. med.*, **44**(10), 53-56 (1957)
91. Il'ina, L. I., Blokhina, V. D., and Uspenskaya, M. S., *Med. Radiol. Moskva*, **2**(4), 25-30 (1957)
92. Kritskii, G. A., *Biokhimiya*, **23**, 87-91 (1958)
93. Kuzin, A. M., and Budilova, E. V., *Doklady Akad. Nauk S.S.S.R.*, **91**, 1183-86 (1953)
94. Luchnik, N. V., *Biokhimiya*, **21**, 668-70 (1956)
95. Manollov, S. E., Nemchinskaya, V. L., Alieva, A. Z., and Mytareva, L. V., *Biokhimiya*, **22**, 1013-18 (1957)
96. Gintsburg, M. B., Pandre, E. M., and Binus, N. M., *Biokhimiya*, **22**, 467-75 (1957)
97. Braunshteln, A. E., and Azarkh, R. M., *Voprosy Med. Khim.*, **3**, 380-92 (1957)
98. Yakovleva, T. M., *Doklady Akad. Nauk S.S.S.R.*, **89**, 347-50 (1953)
99. Bresler, S. E., and Rubina, Kh. M., *Biokhimiya*, **20**, 740-48 (1955)
100. Vul'fson, P. L., *Biokhimiya*, **20**, 179-87 (1955)
101. Lessakova, A. S., *Akusherstvo i Ginekol.*, **1955**(6), 3-6
102. Kuzin, A. M., Garzunova, G. A., and Mamul, Ya. V., *Doklady Akad. Nauk S.S.S.R.*, **92**, 637-40 (1953)
103. Stepanenko, B. N., and Afanas'eva, E. M., *Doklady Akad. Nauk S.S.S.R.*, **90**, 1095-98 (1953)
104. Afanas'eva, E. M., and Stepanenko, B. N., *Biokhimiya*, **21**, 603-11 (1956)
105. Shubich, M. E., *Arkh. Anat., Gistol. i Embriol.*, **33**(3), 32-34 (1956)
106. Kanashenok, P. S., *Biull. eksptl. biol. med.*, **38**(10), 45-48 (1954)
107. Epel'baum, S. E., and Kantor, L. F., *Biokhimiya*, **19**, 660-65 (1954)
108. Ferdman, D. L., *Voprosy Med. Khim.*, **3**, 351-66 (1957)
109. Sheves, G. S., *Biokhimiya*, **18**, 63-70 (1953)
110. Telepneva, V. I., *Biokhimiya*, **20**, 212-19 (1955)
111. Slozhenikina, L. V., *Voprosy Med. Khim.*, **3**, 137-49 (1957)
112. Kanashenok, P. S., *Biull. eksptl. biol. med.*, **37**(4), 40-42 (1954)
113. Tsinkalovs'ka, S. M., *Ukrain. Biokhim. Zhur.*, **29**, 458-68 (1957)
114. Kakhana, M. S., and Telenkevich, A. E., *Biull. eksptl. biol. med.*, **37**(4), 23-25 (1954)

115. Kanashenok, P. S., *Biull. eksptl. biol. med.*, **38**(9), 36-38 (1954)
116. Skvortsova, R. I., *Biokhimiya*, **18**, 594-602 (1953)
117. Razina, L. G., *Biull. eksptl. biol. med.*, **43**(5), 87-91 (1957)
118. Meshkova, N. P., and Zaitseva, N. N., *Doklady Akad. Nauk S.S.S.R.*, **92**, 999-1002 (1953)
119. Nagradova, N. K., *Biokhimiya*, **21**, 17-25 (1956)
120. Severin, S. E., and Meshkova, N. P., *Doklady Akad. Nauk S.S.S.R.*, **92**, 807-10 (1953)
121. Dikanova, A. A., *Biokhimiya*, **20**, 414-19 (1955)
122. Chernyak, N. B., *Voprosy Med. Khim.*, **3**, 218-27 (1957)
123. Luganova, I. S., Sefts, I. F., and Teodorovich, V. I., *Doklady Akad. Nauk S.S.S.R.*, **118**, 537-39 (1958)
124. Luganova, I. S., Sefts, I. F., and Teodorovich, V. I., *Voprosy Med. Khim.*, **3**, 428-39 (1957)
125. Vladimirov, G. E., Ashmarin, I. P., and Urinson, A. P., *Biokhimiya*, **18**, 582-93 (1953)
126. Venkstern, T. V., and Engel'gardt, V. A., *Doklady Akad. Nauk S.S.S.R.*, **102**, 133-36 (1955)
127. Chirkov, V. V., *Terap. Arkh.*, **27**(6), 21-23 (1955)
128. Khaes, S. I., *Farm. Toks., Moskva*, **17**(4), 58-59 (1954)
129. Khaes, S. I., *Voprosy Pitaniya*, **14**(2), 13-16 (1955)
130. Petrova, A. N., *Problemy Endokrinol. i Gormonoterap.*, **1**(3), 80-85 (1955)
131. Petrova, A. N., and Bekina, R. M., *Biokhimiya*, **21**, 368-73 (1956)
132. Petrova, A. N., *Biokhimiya*, **20**, 718-24 (1955)
133. Bekina, R. M., and Petrova, A. N., *Biokhimiya*, **20**, 444-49 (1955)
134. Petrova, A. N., *Biokhimiya*, **19**, 24-29 (1954)
135. Stepanenko, B. N., Zubrilina, G. V., and Khayurova, L. P., *Doklady Akad. Nauk S.S.S.R.*, **100**, 521-24 (1955)
136. Smirnov, N. P., *Arkh. Patol.*, **16**, 33-42 (1954); *Problemy Endokrinol. i Gormonoterap.*, **3**(1), 10-19 (1957)
137. Veller, N. S., and Charnaya, P., *Arkh. Patol.*, **17**(7), 63 (1955)
138. Leites, S. M., Pavlov, G. T., and Yakusheva, T. S., *Fiziol. Zhur. S.S.S.R.*, **41**, 249-56 (1955)
139. Idel'chik, E. I., *Biull. eksptl. biol. med.*, **37**(7), 46-49 (1954)
140. Il'in, V. S., and Titova, G. V., *Biull. eksptl. biol. med.*, **42**(9), 33-36 (1956)
141. Pravotorova, E. L., *Biull. eksptl. biol. med.*, **42**(8), 59-61 (1956)
142. Tseftlin, L. A., *Biokhimiya*, **20**, 725-29 (1955)
143. Gruzdeva, K. N., *Biull. eksptl. biol. med.*, **42**(12), 40-42 (1956)
144. Karyakina, V. A., *Biokhimiya*, **20**, 377-80 (1955)
145. Kanfor, I. S., *Biull. eksptl. biol. med.*, **39**(6), 7-10 (1955)
146. Bannikova, N. A., *Fiziol. Zhur. S.S.S.R.*, **43**, 1176-82 (1957)
147. Sergeeva, M. A., *Voprosy Pitaniya*, **15**(1), 9-15 (1956)
148. Graevskaya, B. M., and Kellina, R. Ya., *Vestnik Rentgenol. i Radiol.*, **30**(4), 21-26 (1955)
149. Blokhin, N. N., Luganova, I. S., and Rotfel'd, L. S., *Med. Radiol., Moskva*, **1**(6), 40-46 (1956)
150. Toropova, G. P., *Voprosy Pitaniya*, **17**(1), 50-54 (1958)
151. Kellina, R. Ya., *Biokhimiya*, **20**, 420-24 (1955)
152. Prokudina, E. A., *Med. Radiol., Moskva*, **1**(6), 46-51 (1956)
153. Kellina, R. Ya., and Komarov, E. I., *Fiziol. Zhur. S.S.S.R.*, **44**, 148-52 (1958)

154. Bavina, M. V., and Kritsman, M. G., *Doklady Akad. Nauk S.S.S.R.*, **88**, 313-16 (1953)
155. Tarasova, L. S., *Voprosy Med. Khim.*, **3**, 177-82 (1957)
156. Kritsman, M. G., and Bavina, M. V., *Doklady Akad. Nauk S.S.S.R.*, **94**, 721-24 (1954)
157. Kritsman, M. G., Gavrilova, K. I., and Konikova, A. S., *Biokhimiya*, **19**, 557-60 (1954)
158. Alekseeva, A. S., *Biull. eksptl. biol. med.*, **41**(2), 39-41 (1956)
159. Bavina, M. V., and Alekseeva, A. S., *Biull. eksptl. biol. med.*, **41**(3), 49-53 (1956)
160. Areshkina, L. Ya., Bukin, V. N., Erofeeva, N. N., and Skorobogatova, E. P., *Biokhimiya*, **22**, 384-90 (1957)
161. Leutskii, K. M., and Lyubovich, E. N., *Doklady Akad. Nauk S.S.S.R.*, **96**, 341-42 (1954)
162. Kruchakova, F. A., *Voprosy Med. Khim.*, **3**, 183-89 (1957)
163. Kurdybaflo, F. V., *Biull. eksptl. biol. med.*, **41**(2), 42-45 (1956)
164. Shepshelevich, L. L., *Pat. fiziol., Moskva*, **2**(1), 27-33 (1958)
165. Fokina, T. V., *Biull. eksptl. biol. med.*, **43**(4), 49-53 (1957)
166. Makarova, A. R., *Fiziol. Zhur. S.S.S.R.*, **42**, 225-31 (1956)
167. Zhmakin, I. K., *Biull. eksptl. biol. med.*, **43**(5), 39-42 (1957)
168. Isaakyan, L. A., *Fiziol. Zhur. S.S.S.R.*, **41**, 210-18 (1955)
169. Danetskaya, E. V., *Biull. eksptl. biol. med.*, **44**(8), 63-66 (1957)
170. Skvirskaya, E. B., and Chepinoga, O. P., *Doklady Akad. Nauk S.S.S.R.*, **92**, 1007-10 (1953)
171. Manukyan, K. G., *Doklady Akad. Nauk S.S.S.R.*, **102**, 567-70 (1955)
172. Manukyan, K. G., *Doklady Akad. Nauk S.S.S.R.*, **101**, 1085-88 (1955)
173. Verzhbinskaya, N. A., *Fiziol. Zhur. S.S.S.R.*, **39**, 17-26 (1953)
174. Lisovskaya, N. P., *Biokhimiya*, **19**, 626-37 (1954)
175. Sadikova, N. V., and Skvortsevich, V. A., *Voprosy Med. Khim.*, **2**, 128-32 (1956)
176. Vladimirov, G. E., Ivanova, T. N., and Pravdina, N. I., *Biokhimiya*, **21**, 155-62 (1956)
177. Grodzenskiĭ, D. E., and Avakyan, A. A., *Biull. eksptl. biol. med.*, **37**(7), 37-41 (1954)
178. Savich, K. V., and Yakovlev, V. A., *Voprosy Med. Khim.*, **3**, 121-28 (1957)
179. Panchenko, L. F., *Fiziol. Zhur. S.S.S.R.*, **44**, 243-48 (1958)
180. Dmitriev, V. F., *Biokhimiya*, **20**, 527-32 (1955)
181. Palladin, A. V., Polyakova, N. M., and Silich, T. P., *Ziziol. Zhur. S.S.S.R.*, **43**, 611-18 (1957)
182. Khor'kova, N. N., *Biokhimiya*, **21**, 709-14 (1956)
183. Skvirskaya, E. B., and Silich, T. P., *Doklady Akad. Nauk S.S.S.R.*, **93**, 1073-75 (1953)
184. Golubtsova, A. V., Moiseenko, E. V., and Safronova, M. I., *Biull. eksptl. biol. med.*, **42**(9), 36-39 (1956)
185. Engel'gardt, V. A., and Lisovskaya, N. P., *Biokhimiya*, **20**, 225-35 (1955)
186. Nechaeva, G. A., *Biokhimiya*, **22**, 546-53 (1957)
187. Palladin, A. V., Belik, Ya. V., and Krachko, L. I., *Biokhimiya*, **22**, 359-68 (1957)
188. Vladimirov, G. E., and Urinson, A. P., *Biokhimiya*, **22**, 709-14 (1957)
189. Smirnov, A. A., and Chetverikov, D. A., *Doklady Akad. Nauk S.S.S.R.*, **90**, 843-45 (1953)

190. Gershenovich, Z. S., and Krichevskaya, A. A., *Doklady Akad. Nauk S.S.S.R.*, **95**, 837-40 (1954); *Biokhimiya*, **21**, 715-22 (1956)
191. Vladimirova, E. A., *Doklady Akad. Nauk S.S.S.R.*, **90**, 1191-94 (1953)
192. Baranov, M. N., *Biokhimiya*, **22**, 830-37 (1957)
193. Smirnov, A. A., and Chetverikov, D. A., *Doklady Akad. Nauk S.S.S.R.*, **90**, 631-33 (1953)
194. Verzhbinskaya, N. A., and Volkova, R. I., *Doklady Akad. Nauk S.S.S.R.*, **118**, 135-38 (1958)
195. Volkova, R. I., *Biokhimiya*, **22**, 645-50 (1957)
196. Savchenko, O. N., *Biokhimiya*, **22**, 702-8 (1957)
197. Malkiman, I. I., *Biull. eksptl. biol. med.*, **37**(4), 14-17 (1954)
198. Smirnov, A. A., *Doklady Akad. Nauk S.S.S.R.*, **101**, 913-16 (1955)
199. Prokhorova, M. I., Brodskaya, N. I., and Sokolova, G. P., *Voprosy Med. Khim.*, **3**, 279-85 (1957)
200. Shuster, M. I., *Arkh. Patol.*, **15**(2), 55-60 (1953)
201. Petrov, I. R., Rafko, Z. A., and Kudritskaya, T. E., *Fiziol. Zhur. S.S.S.R.*, **43**, 107-16 (1957)
202. Yakovlev, N. N., *Voprosy Med. Khim.*, **2**, 140-49 (1956)
203. Gershenovich, Z. S., and Bronovitskaya, Z. G., *Biokhimiya*, **20**, 425-30 (1955)
204. Ivanov, V. I., and Rozenberg, P. A., *Biull. eksptl. biol. med.*, **39**(2), 33-35 (1955)
205. Palladin, A. V., and Rybina, A. A., *Doklady Akad. Nauk S.S.S.R.*, **91**, 903-5 (1953)
206. Khalkina, B. I., and Goncharova, E. E., and Mikhailovskaya, L. A., *Doklady Akad. Nauk S.S.S.R.*, **96**, 347-49 (1954)
207. Vladimirova, E. A., *Voprosy Med. Khim.*, **2**, 47-52 (1956)
208. Sytinskiĭ, I. A., *Biokhimiya*, **21**, 359-67 (1956)
209. Maev'ska, I. P., *Fiziol. Zhur., Akad. Nauk Ukr. R.S.R.*, **3**(4), 101-5 (1957)
210. Nedzvetskiĭ, S. V., and Ratnitskaya, S. S., *Biokhimiya*, **19**, 677-82 (1954)
211. Polyakova, N. M., *Doklady Akad. Nauk S.S.S.R.*, **93**, 321-24 (1953)
212. Leutskiĭ, K. M., *Vitaminy, Akad. Nauk Ukr.*, **2**, 144-51 (1956)
213. Vladimirov, G. E., Ivanova, T. N., and Pravdina, N. I., *Biokhimiya*, **19**, 578-85 (1954); **22**, 351-58 (1957)
214. Trufanov, A. V., and Popova, G. M., *Biokhimiya*, **21**, 3-9 (1956)
215. Savitskiĭ, I. V., *Fiziol. Zhur., Akad. Nauk Ukr. R.S.R.*, **4**, 121-30 (1958)
216. Matskevich, M. S., and Mamul, Ya. V., *Doklady Akad. Nauk S.S.S.R.*, **88**, 733-36 (1953)
217. Kashchenko, L. A., *Problemy Endokrinol. i Gormonoterap.*, **1**(3), 65-68 (1955)
218. Siver, P. Ya., Zamanskiĭ, L. N., and Lopushanskiĭ, A. I., *Biull. eksptl. biol. med.*, **39**(6), 43-45 (1955)
219. Epel'baum, S. E., and Dergorisova, E. A., *Biokhimiya*, **21**, 491-95 (1956)
220. Kovalenko, T. M., *Arkh. Anat. Gistol. i Embriol.*, **34**(1), 22-28 (1957)
221. Azyavchik, A. V., *Biokhimiya*, **18**, 324-28 (1953)
222. Gol'dshteln, B. I., and Gotovtseva, E. P., *Biokhimiya*, **22**, 994-99 (1957)
223. Potop, I., *Biokhimiya*, **23**, 11-15 (1958)
224. Mandl, S. F., *Problemy Endokrinol. Gormonoterap.*, **2**(1), 37-41 (1956)
225. Alekperov, M. A., *Problemy Endokrinol. Gormonoterap.*, **1**(6), 34-36 (1955)
226. Epova, I. E., *Biull. eksptl. biol. med.*, **43**(2), 80-84 (1957)
227. Alov, I. A., *Biull. eksptl. biol. med.*, **40**(12), 47-50 (1955)
228. Voltkevich, A. A., Sibkina, M. Ya., Khomullo, G. V., Gordina, S. N., Munalt-

- basova, G. A., Tukaeva, S. N., Negovskaya, A. V., and Smirnov, I. P., *Problemy Endokrinol. Gormonoterap.*, **1**(2), 20-25 (1955)
229. Genes, S. G., and Lesnof, N. G., *Problemy Endokrinol. Gormonoterap.*, **2**(3), 38-48 (1956)
230. Krushinskii, L. V., and Dobrokhotova, L. P., *Biull. eksptl. biol. med.*, **44**(8), 46-49 (1957)
231. Rodkina, B. S., *Problemy Endokrinol. Gormonoterap.*, **2**(4), 101-9 (1956)
232. Utevskii, A. M., and Butom, M. L., *Biokhimiya*, **21**, 776-83 (1956)
233. Vunder, P. A., *Problemy Endokrinol. Gormonoterap.*, **3**(4), 30-35 (1957)
234. Mitskevich, M. S., *Zhur. Obshchei Biol.*, **15**, 115-27 (1954)
235. Kolli, E. A., *Biokhimiya*, **19**, 273-79 (1954)
236. Aleshin, B. V., and Demidenko, N. S., *Med. Radiol., Moskva*, **2**(3), 77-82 (1957)
237. Tarakanov, E. I., *Problemy Endokrinol. Gormonoterap.*, **1**(5), 75-84 (1955)
238. Amiragova, M. G., *Doklady Akad. Nauk S.S.S.R.*, **99**, 325-28 (1954)
239. Arkhipenko, V. I., *Problemy Endokrinol. Gormonoterap.*, **2**(1), 42-50 (1956)
240. Votkevich, A. A., *Doklady Akad. Nauk S.S.S.R.*, **95**, 1125-28 (1954)
241. Vunder, P. A., *Problemy Endokrinol. Gormonoterap.*, **1**(2), 15-20 (1955)
242. Skebel'skaya, Yu. B., *Problemy Endokrinol. Gormonoterap.*, **1**(2), 9-15 (1955)
243. Isichenko, N. A., *Problemy Endokrinol. Gormonoterap.*, **1**(4), 89-95 (1955)
244. Amiragova, M. G., *Biull. eksptl. biol. med.*, **45**(2), 7-12 (1958)
245. Amiragova, M. G., *Biull. eksptl. biol. med.*, **44**(10), 33-38 (1957)
246. Gincherman, E. Z., and Ioffe, B. M., *Problemy Endokrinol. Gormonoterap.*, **1**(3), 71-75 (1955)
247. Rabkina, A. E., *Problemy Endokrinol. Gormonoterap.*, **1**(6), 16-24 (1955)
248. Baranov, V. G., Speranskaya, E. N., and Tendler, D. S., *Problemy Endokrinol. Gormonoterap.*, **1**(1), 28-33 (1955); *Biull. eksptl. biol. med.*, **39**(6), 3-7 (1955)
249. Vasil'eva, A. G., *Problemy Endokrinol. Gormonoterap.*, **1**(3), 46-65 (1955)
250. Genes, S. G., and Lesnof, N. G., *Biull. eksptl. biol. med.*, **43**, Suppl. 1, 113-17 (1957)
251. Dzogoeva, T. O., *Fiziol. Zhur., Akad. Nauk Ukr. R.S.S.R.*, **4**(1), 90-96 (1958)
252. Utevskii, A. M., and Butom, M. L., *Biokhimiya*, **18**, 195-200 (1953)
253. Ozerova, M. R., *Problemy Endokrinol. Gormonoterap.*, **1**(4), 54-59 (1955)
254. Gershenovich, Z. S., Krichevskaya, A. A., and Alekseenko, L. P., *Ukrain. Biokhim. Zhur.*, **27**(1), 3-11 (1955)
255. Emel'yanova, A. V., *Fiziol. Zhur. S.S.S.R.*, **40**, 53-59 (1954)
256. Osinskaya, V. O., *Biokhimiya*, **22**, 537-45 (1957)
257. Barts, M. P., *Biokhimiya*, **22**, 677-85 (1957)
258. Barts, M. P., *Problemy Endokrinol. Gormonoterap.*, **3**(2), 33-39 (1957)
259. Poskalenko, A. N., *Problemy Endokrinol. Gormonoterap.*, **1**(5), 93-96 (1955)
260. Sveshnikova, N. A., *Biull. eksptl. biol. med.*, **44**(7), 54-59 (1957)
261. Karagezyan, K. G., *Doklady Akad. Nauk S.S.S.R.*, **118**, 142-45 (1958)
262. Pashchenko, A. E., *Problemy Endokrinol. Gormonoterap.*, **3**(1), 25-30 (1957); **3**(5), 74-81 (1957)
263. Il'ina, A. I., and Tonkikh, A. V., *Fiziol. Zhur. S.S.S.R.*, **43**, 3-13 (1957)
264. Mikhailova, N. V., *Problemy Endokrinol. Gormonoterap.*, **1**(1), 59-64 (1955)
265. Trachik, V., *Problemy Endokrinol. Gormonoterap.*, **3**(3), 25-34 (1957)
266. Anichkov, S. V., *Fiziol. Zhur. S.S.S.R.*, **43**, 685-90 (1957)
267. Mikhailova, N. V., *Problemy Endokrinol. Gormonoterap.*, **2**(5), 9-12 (1956)
268. Isichenko, N. A., *Problemy Endokrinol. Gormonoterap.*, **2**(5), 19-24 (1956)
269. Pyshina, S. P., *Fiziol. Zhur. S.S.S.R.*, **42**, 931-38 (1956)

270. Nikolov, N. A., *Fiziol. Zhur. S.S.S.R.*, **42**, 925-30 (1956)
271. Stroganova, E. V., *Problemy Endokrinol. Gormonoterap.*, **1**(4), 103-14 (1955)
272. Eskin, I. A., *Pat. fiziol., Moskva*, **1**(4), 10-15 (1957)
273. Yudaev, N. A., and Druzhinina, K. V., *Problemy Endokrinol. Gormonoterap.*, **2**(5), 3-8 (1956)
274. Guillemin, R., and Rosenberg, B., *Endocrinology*, **57**, 599-607 (1955)
275. Mednik, G. L., *Problemy Endokrinol. Gormonoterap.*, **3**(6), 36-39 (1957)
276. Yudaev, N. A., Lebedev, M. B., and Zavval'skaya, N. P., *Problemy Endokrinol. Gormonoterap.*, **3**(6), 13-21 (1957)
277. Protasova, T. N., *Biokhimiya*, **18**, 89-96 (1953)
278. Seifulla, Kh. I., *Farm. toks., Moskva*, **19**, Suppl., 33-35 (1956)
279. Smirnov, N. P., *Problemy Endokrinol. Gormonoterap.*, **1**(4), 81-88 (1955)
280. Serdyukova, O. A., and Yusfina, E. Z., *Problemy Endokrinol. Gormonoterap.*, **1**(5), 97-104 (1955)
281. Mednik, G. L., *Problemy Endokrinol. Gormonoterap.*, **3**(3), 61-63 (1957)
282. Yudaev, N. A., Pankov, Yu. A., and Surikova, N. P., *Problemy Endokrinol. Gormonoterap.*, **3**(1), 20-24 (1957)
283. Skebel'skaya, Yu. B., *Problemy Endokrinol. Gormonoterap.*, **3**(6), 32-35 (1957)
284. Eskin, I. A., *Problemy Endokrinol. Gormonoterap.*, **1**(1), 52-59 (1955)
285. Leites, S. M., and Yakusheva, T. S., *Problemy Endokrinol. Gormonoterap.*, **1**(6), 47-56 (1955)
286. Rabkina, A. E., *Problemy Endokrinol. Gormonoterap.*, **2**(5), 25-31 (1956)
287. Veller, N. S., and Charnaya, P. M., *Problemy Endokrinol. Gormonoterap.*, **2**(5), 44-49 (1956)
288. Tret'yakova, K. A., *Problemy Endokrinol. Gormonoterap.*, **3**(3), 72-74 (1957)
289. Bozhenko, L. V., *Med. Radiol., Moskva*, **2**(4), 44-51 (1957)
290. Zhorno, L. Ya., *Med. Radiol., Moskva*, **1**(1), 79-87 (1956)
291. Pavlova, E. B., and Rabkina, A. E., *Problemy Endokrinol. Gormonoterap.*, **3**(4), 3-9 (1957)
292. Orlova, L. V., Rodionov, V. M., with Chekmenev, I. L., *Pat. fiziol., Moskva*, **1**(4), 22-26 (1957)
293. Katorovich, I. N., *Fiziol. Zhur. S.S.S.R.*, **40**, 697-703 (1954)
294. Mityushov, M. I., *Problemy Endokrinol. Gormonoterap.*, **1**(1), 84-92 (1955)
295. Veller, N. S., Genes, S. G., Rodkina, B. S., and Charnaya, P. M., *Problemy Endokrinol. Gormonoterap.*, **1**(1), 77-84 (1955)
296. Yankelevich, D. E., *Problemy Endokrinol. Gormonoterap.*, **1**(3), 75-80 (1955)
297. Shorm, F., and Shveltsar, Yu., *Biokhimiya*, **20**, 241-48 (1955)
298. Makarevich-Gal'perin, L. M., and Ushenko, S. N., *Problemy Endokrinol. Gormonoterap.*, **2**(2), 95-102 (1956)
299. Novikova, N. V., and Kuz'menko, I. I., *Problemy Endokrinol. Gormonoterap.*, **2**(2), 103-8 (1956)
300. Genes, S. G., *Fiziol. Zhur. S.S.S.R.*, **43**, 461-68 (1957)
301. Plavskaya, A. A., *Problemy Endokrinol. Gormonoterap.*, **3**(3), 52-60 (1957)
302. Alov, I. A., *Arkh. Anat. Gistol. i Embriol.*, **34**(6), 75-79 (1957)
303. Khrustaleva, G. F., *Biull. eksptl. biol. med.*, **43**, Suppl. 1, 126-29 (1957)
304. Isichenko, N. A., *Problemy Endokrinol. Gormonoterap.*, **1**(2), 67-73 (1955)
305. Vartapetov, B. A., Kalmykova, K. M., and Sudakova, A. D., *Problemy Endokrinol. Gormonoterap.*, **1**(2), 85-89 (1955)
306. Fel'berbaum, I. M., *Problemy Endokrinol. Gormonoterap.*, **1**(4), 73-78 (1955)
307. Samtsova, A. V., *Problemy Endokrinol. Gormonoterap.*, **3**(2), 6-11 (1957)

308. Blagosklonnaya, Ya. V., *Problemy Endokrinol. Gormonoterap.*, 3(1), 88-95 (1957)
309. Kashchenko, L. A., and Pushnitsyna, A. D., *Doklady Akad. Nauk S.S.S.R.*, 88, 575-77 (1953)
310. Kashchenko, L. A., and Pushnitsyna, A. D., *Vestnik Rentgenol. i Radiol.*, 31(4), 3-11 (1956)
311. Benetato, G., Oprishiu, K., Tudorash, T., and Kon-Derevenko, V., *Problemy Endokrinol. Gormonoterap.*, 3(2), 26-32 (1957)
312. Kharvat, I., and Golechek, V., *Problemy Endokrinol. Gormonoterap.*, 3(2), 17-25 (1957)
313. Kakhana, M. S., *Problemy Endokrinol. Gormonoterap.*, 3(4), 50-57 (1957)
314. Ryabushko, E. O., *Problemy Endokrinol. Gormonoterap.*, 3(5), 82-88 (1957)

REPRODUCTION^{1,2}

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Because so many reports on reproductive physiology appear each year, it is impossible to cover the entire subject in one review. Consequently, the present account primarily summarizes studies on the physiology of hormonal relations involved in the reproduction of the mammal, with special reference to man.

OXYTOCIN

Determination.—The method for extracting oxytocin from blood has been improved giving recoveries of 77 per cent (1, 2). The sensitiveness and accuracy of the isolated rat uterus method of assay have been increased (3, 4, 5). The rabbit uterus, *in vivo* after mid-thoracic section of the spinal cord, loses spontaneous motility and becomes a very sensitive test for oxytocin (threshold dose 1 to 5 mU) (6). Its response to ADH is qualitatively different (biphasic) and similar to that produced by epinephrine (6). The successful induction of labor in rabbits on the 31st day of pregnancy has been used as a test for oxytocin and other oxytocic substances (7, 8). The cat uterus *in situ* and *in vitro* has also been employed (7), and an increasing number of investigators (6, 9, 10, 11, 14, 47, 50) are using the milk-ejecting action on the rabbit.

Synthetic analogues of oxytocin.—By replacing the isoleucyl group of oxytocin with phenylalanyl, leucyl, or valyl residues, three analogues (referred to as P, L, and V) were obtained (12, 13).

The dose of L and V analogues which produces on the arterial pressure of the chicken (U.S.P. test) and on the isolated rat uterus (B.P. test) effects similar to those of 3 units of oxytocin has, on the milk ejection test and on the cat uterus *in situ*, effects equivalent to those of 15 units of oxytocin (7, 8, 14).

In the pregnant human uterus, the same dose of V-analogue has an effect

¹ The survey of literature pertaining to this review was concluded in June, 1958.

² The following abbreviations are used in this chapter: FSH (follicular stimulating hormone); LH (luteinizing hormone); LTH (luteotrophic hormone); PMS (pregnant mare's serum); ACTH (adrenocorticotrophic hormone); HCG (human chorionic gonadotrophin); ICSH (interstitial cell stimulating hormone); ADH (antidiuretic hormone); mU (milliunits).

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similar to that of six units of oxytocin (15). In other words, the different tests currently used for oxytocin bioassay are not equivalent and give inaccurate information on the effects that a given compound will have on the pregnant human uterus.

The replacement of the asparaginyl group with a glutaminyl residue markedly reduces the oxytocic activity of the molecule (7, 8).

Natural oxytocinlike substance.—Hydrolysis of blood serum or of some of its proteins by certain proteolytic enzymes liberates polypeptides which have oxytocic and antidiuretic effects (16).

Hawker finds a substance similar to oxytocin, but not inactivated by sodium thioglycollate, in the hypothalamus of oxen, cows, cats, rats, and mice (17) and in the human female blood (18) where it increases during suckling (19).

Unity or plurality of neurohypophyseal hormones.—The view that the octapeptides oxytocin and ADH are two hormones existing independently in the neurohypophysis (and not degradation products of a larger protein molecule) has received further support from Acher & Fromageot (20) who showed that in the rat: (a) the oxytocic and the vasopressor activities of the active protein extracted from the neurohypophysis behave towards chemical and enzymatic agents exactly like the activities of the pure octapeptides; (b) processes which do not involve hydrolysis (such as electro-dialysis), dissociate the active protein into substances responsible for the oxytocic and vasopressor activities; (c) the vasopressor/oxytocic ratio in the neurohypophysis of the rat increases during lactation (a result of a reduction of the amounts of the oxytocic principle) suggesting an independent release of oxytocin. The depletion of oxytocin has been confirmed in lactating dogs (21).

Van Dyke's (21) present view is that in oxen a protein containing the polypeptides is stored in the posterior lobe.

Oxytocin in the hypothalamus and neurohypophysis.—The evidence concerning the endocrine function of the neurohypophysis and its nervous control is reviewed by Dale (22). It is currently accepted (21) that the neurons of the paraventricular and supraoptic nuclei secrete oxytocin and ADH which migrate in or along their axones and are finally stored in the posterior lobe. Further evidence of neurosecretion has been found in adult mammals (23, 24, 25), in the cow embryo from the third month of pregnancy (26), in toads (27), and in invertebrates (28). Hypophysectomy increases the oxytocic effect of hypothalamic extracts (29).

In most species, only 1 per cent or less of the total oxytocin is found in the hypothalamus, the remaining 99 per cent or more being stored in the posterior lobe of the pituitary. The only known exception is the dog, which may have up to 8 per cent of the total oxytocin in the hypothalamus (21). The contents of oxytocin of the hypothalamus and the neurohypophysis cannot be accepted as a reliable indication of hormone release because they depend on the equilibrium between rate of repletion and rate of release (30). In lactating dogs (21) oxytocin and ADH are depleted from the posterior

lobe, the depletion of oxytocin being the greater (10,500 mU in the normal dog; 4,300 mU in the lactating dog). A similar depletion of oxytocin has been observed in rats during parturition and lactation (20).

Heller (3) & Lederis found that the pituitary of rats in metoestrus contained significantly less oxytocin and ADH than the glands of animals in diestrus or oestrus. An oxytocinlike substance has been found in the adenohypophysis of rat and oxen (31, 32).

Release of oxytocin.—Whereas the role of oxytocin in lactation is widely accepted, there is still some controversy in regard to its functions during mating and parturition (5).

In cats, electrical stimulation of the amygdaloid nucleus increases uterine contractility (33). The effect is more marked in the pregnant cat where it induces labor. Spinal section at T6 enhances the response of the uterus to the amygdaloid stimulation (33), which very possibly acts through release of oxytocin.

In rabbits electrical stimulation of the paraventricular or supraoptical nuclei and of the hypothalamohypophysial nerve pathway to the tuber cinereum and neural stalk produces effects similar to the intravenous injection of 10 to 50 mU of oxytocin, both in the uterus and in the mammary gland (6). These effects are not prevented by spinal section or epidural anesthesia of the cord. Labor is induced by hypothalamic stimulation (9) showing that endogenous oxytocin can initiate the process of birth.

In nonpregnant, spayed, estrogenized rabbits, Cross (6) has not been able to increase the contractility of one uterine horn by distending the uterine cervix of the other horn, thus failing to confirm Ferguson's classical findings in the post-partum rabbit. On the contrary, the same author (9) records milk-ejecting effect during parturition which he attributes to a release of oxytocin reflexly produced by distention of the uterus and cervix. In the spontaneous parturition of the sow, the alveoli of the mammary gland are also emptied of their contents (34). Fitzpatrick (5) reports increased levels of an oxytocinlike substance during manual stimulation of the uterus and cervix in the nonpregnant cow. Hawker *et al.* find increased blood levels of oxytocin during milking in cows and goats (35) but not in women (36) (see Table I).

It appears that in many conditions oxytocin and ADH are liberated simultaneously although the stimulus evoking their release requires (teleologically) only one hormone (21). The proportion of oxytocin released is very much higher despite the fact that vasopressin may be the hormone required.

In the rat, arterial hypotension (caused by hemorrhage) produces a great release of ADH (30), whereas intracardial injection of hypertonic solution of sodium chloride failed to evoke any antidiuretic effect (30), as it does in the dog. A possible explanation of this failure is that a predominant release of oxytocin (which has a diuretic effect on the water loaded rat) may have masked the antidiuretic effect of the released ADH.

In pregnant women under water diuresis, the intravenous injection of

large doses of hypertonic solution of sodium chloride produced both marked oxytocic and antidiuretic effects (37).

The release of oxytocin and ADH by the action of nicotine is not blocked by hexamethonium (38, 39) suggesting that the synapse of the supraoptic nuclei has pharmacological properties quite different from those of peripheral autonomic ganglia.

It has been estimated (40, 41, 62) that the secretion rate of oxytocin during parturition in women should be between 2 and 8 mU per min. (see below).

Blood levels of exogenous oxytocin required for stimulating the uterus and the myoepithelial cells of the mammary gland.—During labor, induced with continuous intravenous infusion of synthetic oxytocin, it has been estimated (41, 42) that the human blood levels of oxytocin lie in the range of 20 to 40 micro units per ml. of plasma. This low concentration (which represents 2.3×10^{10} molecules per ml.) is sufficient to produce strong uterine contractions indistinguishable from those of normal spontaneous labor. The pregnant human uterus is undoubtedly very sensitive to oxytocin. Higher concentrations produce abnormal uterine hypercontractility and hypertonicity. The estimations were based on the assumption that all the oxytocin was contained in the intravascular compartment, in agreement with Heller's (3) findings that synthetic oxytocin binds with plasma protein. If oxytocin diffuses freely to the interstitial fluid, its concentration should be much lower.

These figures for oxytocin blood level are in good agreement with those presented by Fitzpatrick (5) for the human and the cow, which have been calculated from the uterine effects of single injections of oxytocin. The results of Cross (6, 9) indicate that similar blood levels of exogenous oxytocin are required in the rabbit to stimulate uterine contractions and milk ejection. In the sow (34), 1 mU per ml. has been estimated as the threshold concentration required in the blood for stimulating the myoepithelial cells of the mammary gland. According to Heller (3) the levels of ADH required for a normal water balance are of a comparable range.

Blood levels of "endogenous oxytocin".—Table I shows the values reported during the last year. There are wide discrepancies in spite of the fact that all of them were obtained with the same methods for extracting the blood and assaying oxytocin. The author (1) of the extraction method recognizes that only one-third of the oxytocin added to the blood is recovered and that there are wide variations in the recovery percentage. The presence in the blood of substances (other than oxytocin) capable of stimulating the test organ (18, 35, 36) and the formation or liberation of other oxytocic substances during the process of extraction (16, 43, 44) could be the cause of the relatively high values found for "endogenous oxytocin" by several authors (2, 5, 18, 35, 38, 39). The reviewers have very little doubt that in a pregnant woman the presence of 900 to 43,000 μ U of oxytocin per ml. of blood (18) would produce a marked uterine hypercontractility, incompatible with the normal maintenance of gestation. The high concentration found by

TABLE I
CONCENTRATION OF "ENDOGENOUS OXYTOCIN" IN VENOUS BLOOD

Species	Condition	Anesthesia	Source of venous blood	Oxytocin blood level microunits per ml.		Stimulus	Reference
				Nonstimulated subjects	Stimulated subjects		
Cow	Nonpregnant (estrous)	None	Jugular	120-300	420-800	Manual distention of uterus and cervix	(5)
Cow	Lactating	None	Jugular	18	250	Milking	(35)
Goat	Lactating	None	Jugular	0	380 ⁵¹	Milking	(35)
Rat	Nonpregnant	Ethanol	Jugular	1100	5,700	Nicotine	(38, 39)
Human male		Barbiturate	Jugular	1200			(2)
Human female	Nonpregnant Pregnant Labor	None None None	Arm Arm Arm	200-7800 950-43,000 100-600			(18)
Human female	Lactating	None	Arm	0-80	0-260	Suckling	(36)

Bisset *et al.* (2) could be caused by the release of oxytocin produced by the barbiturate employed during the recollection of blood.

Oxytocinase and other mechanisms for inactivation or excretion of oxytocin.

—It has been confirmed by several writers (4, 11, 45 to 49) that an enzyme which inactivates oxytocin (natural and synthetic) (41, 46) appears during pregnancy in the blood plasma of primates with hemochorial placentas. The majority of the studies have been made in the human. Semm (4) made a very comprehensive study of oxytocinase including its chemical properties. He found it to be a peptidase which can be separated electrophoretically from the albumin and the alpha-fraction. It is apparently produced by the placenta (4, 10, 45, 48, 50), and its concentration in the plasma of pregnant women increases with the stage of pregnancy, giving maximum values at term and during parturition (4, 11, 41, 48). The only paper (49) claiming that oxytocinase decreases as pregnancy approaches term and is absent during normal labor has since been revised by one of the authors of the paper (51). The high levels of oxytocinase at term pregnancy tend to indicate the importance of oxytocin in parturition (41) according to the view that the "rate of destruction of the hormone indicates its importance in relation to immediate adjustments of the environment" (52).

The blood level of oxytocin during parturition depends on the relation

between: (a) the ability of the body to inactivate oxytocin, and (b) the rate of infusion (or secretion) (41).

At term pregnancy in humans, the plasma inactivates *in vitro*, at 38°, 50 per cent of the added oxytocin in 7 to 9 min. (11, 48). This period coincides very well with the *in vivo* half life of the oxytocin injected to the pregnant woman at term, which has been estimated in 9 min. (41, 53, 54). At mid-pregnancy the lower oxytocinase of the plasma cannot account for the shorter *in vivo* half life of the injected oxytocin, and other mechanisms (renal and hepatic inactivation and excretion of oxytocin) are presumed to be very active (41). Oxytocinase has been demonstrated to occur in the pregnant myometrium, but it is not present in the nonpregnant myometrium (4, 48).

In the rat, the half life of injected oxytocin is 3 min. (55). It increases to 9.7 min. after the renal arteries are clamped. The rat does not have oxytocinase in its plasma. The kidney and liver are probably the main sites of inactivation and excretion of oxytocin, as happens for ADH (56 to 59).

Pregnant human uterus.—The recording of the amniotic fluid pressure and of the intramyometrial pressure showed that the intravenous infusion of oxytocin (at physiological doses—see below) increases the intensity and frequency of uterine contractions without significantly raising the tonus, accelerates the spreading of the contractile waves through the organ, and improves the coordination of the contractions which become identical to those of normal labor (60, 61).

A quantitative relationship has been established between the infusion rate of oxytocin and the response of the uterus (62). In term pregnancies, 2 to 4 mU per min. are usually sufficient to raise uterine contractility to labor values, whereas at mid-pregnancy (20th week) ten times more oxytocin per minute is required for the same purpose. As pregnancy advances, the uterine response to oxytocin increases reaching almost its maximum values at the 32nd to 36th week and showing little change during the last weeks of pregnancy and during labor itself (40, 41, 63).

These results are in general agreement with those of Aburel *et al.* (64) who measure the excitability of the pregnant myometrium by the threshold dose of oxytocin (single intravenous injection) required to produce a contraction detectable by external recorders. The only difference is that Aburel *et al.* find a second rise of uterine excitability occurring during the last two weeks of pregnancy. Using similar methods, Smyth (65) also finds a rise of "uterine irritability" during the days preceding labor. This late increase of uterine response to oxytocin has not been found by the reviewers (40, 41, 60, 61, 63) who administer oxytocin by continuous infusion, and who make quantitative determinations of the spontaneous uterine activity (which increases considerably in the few days preceding labor) and of the increment produced by oxytocin.

After the cessation of the oxytocin infusion, uterine contractility diminishes progressively, returning to the level of the spontaneous activity (53, 54, 62). In mid-pregnancy, this decline of uterine contractility is rather rapid,

being completed in 15 to 25 min. As pregnancy advances, the decline in activity becomes progressively slower until the 36th week, when approximately one hour is required in order to return to the spontaneous activity level (41).

In late pregnancy the infusion of oxytocin at rates higher than 16 mU per min. usually produces excessive uterine contractility (66) and hypertonicity (67) with the consequent reduction in the flow of maternal blood through the uteroplacental vessels and the resultant fetal anoxia (66). Similar effects, but of a short duration, are produced by single rapid intravenous injections of doses higher than 50 mU, which also produce arterial hypotension caused by myocardial weakening (66). The infusion of oxytocin at physiological rates has no other cardiovascular effects than those resulting from the increased uterine contractility (68).

The uterine response to oxytocin infusion is high in toxemia of pregnancy (41, 63) and low in polyhydramnios (69), and also in myxedema (70), where it is restored to normal by treatment with thyroid. It is not changed by chlorpromazine or by meperidine hydrochloride (Demerol) (71, 72). It is not significantly reduced by treatment with large doses of oil progesterone (400 mg. daily during five days) (73) or by acute treatment with hydrosolubilized progesterone (100 mg. intravenously) (74, 75).

Aburel *et al.* (76) find that the oxytocin threshold is decreased by estrogens and very slightly increased by progesterone and testosterone. Petrescu *et al.* (77) claim that it is reduced by exercise and, in abnormal labor, is normalized by artificial rupture of membranes (78).

Rabbit uterus.—The intravenous infusion of oxytocin at rates ranging from 6 to 24 mU per min. produces in the pregnant rabbit at term (6) graded increases of the intensity and frequency of uterine contractions (6), very similar to those obtained in pregnant women (62, 63). Per kilogram of body weight, the human requires approximately 100 times less oxytocin per minute than the rabbit as a threshold dose.

In the rabbit, at the 30th day of pregnancy, the average threshold dose of oxytocin by single injection is 24 mU (79), and a single injection of 100 to 200 mU is sufficient to induce labor in all cases (9). The response to oxytocin is markedly reduced by a previous injection (20 sec. before) of 3 mg. of epinephrine (6).

According to Schofield (79), from the 2nd to the 29th day of pregnancy the rabbit uterus does not respond at all to oxytocin, even if administered in very large doses, that is 5000 mU intravenously. Its behavior is quite different from the human uterus which at mid-pregnancy produces strong contractions in response to infusion rates of 16 mU per min. (40, 41, 60, 61, 63). The rabbit uterus reacquires its responsiveness to oxytocin only in the last two or three days of pregnancy. Whereas, in the human, the major increase in the sensitiveness of the uterus to oxytocin occurs between the 4th and the 8th month of gestation, in the rabbit it does not occur until the very end of pregnancy. These *in vivo* studies confirm the already classic *in vitro* work of

Robson who in 1933 observed similar species differences between the rabbit and the human uterus. These studies are in disagreement with the statement that "certain basic features of myometrial contractility seem to be free of species differences" (80).

Bovine uterus.—At mid-pregnancy the cow requires ten times more oxytocin than just before labor, in order to produce equivalent contractions of the corpus uteri (5). The response of the cervix to oxytocin is similar at both stages of pregnancy, being greater than that of the corpus at mid-pregnancy (favouring the retention of the uterine contents) and becoming smaller before parturition, a pattern which favours the delivery of the fetus. Similar changes of the corpus-isthmus relationships in the response to oxytocin have been found in the human by Hoff (81).

In the nonpregnant cow, the response of the corpus uteri to oxytocin shows no significant variations at different stages of the cycle. The cervix during estrus has a very strong response to oxytocin, dominating that of the corpus, a pattern favourable to the sperm ascension. In the luteal phase, the cervical response to oxytocin becomes low (corpus dominance) (5).

Mouse uterus.—In the mouse, pretreatment with progesterone reduces uterine responsiveness to oxytocin whereas pretreatment with relaxin increases that response, and estrogens are without effect (82, 83).

Mammary gland.—The milk-ejecting effect of oxytocin has been used in rabbits as a test for the quantitative determination of oxytocin (7, 10, 11, 14, 47, 50) and for demonstration of the release of endogenous oxytocin during parturition in rabbits (9) and sow (34). Increased blood levels of oxytocin during milking have been reported in goats and cows (35) but not in women (36). Oxytocin probably stimulates the release of prolactin since it delays the involution of the mammary gland in rats after weaning (84, 85) and increases the production of milk in hypogalactic women (86).

Kidney.—In rats, oxytocin has natriuretic and diuretic effects (44, 87 to 91) which disappear after hypophysectomy and are restored by DCA+NaCl administration (92). In dogs, oxytocin increases the renal plasma flow and, when the urinary flow is low, increases also the excretion of sodium and potassium (93).

GONADOTROPHINS

It has been claimed that there are portions of the molecule which are common to all gonadotrophins (94), that there is only one pituitary gonadotrophin which produces FSH or LH effects according to its blood concentration (95), and that only one molecule is responsible for the two effects of PMS (96).

Secretion.—In women with nervous anorexia, ACTH and cortisone stimulate the secretion of gonadotrophin (97), which is inhibited by high doses of progesterone in menopausal subjects (98). In the rat, LTH secretion is inhibited by chlorpromazine (99) and enhanced by trilaphon (1-(2-hydroxyethyl)-4[3-chloro-10-(phenothiazinyl)-propyl]-piperazine) (100). Blockade

of the release of "pituitary ovulating hormone" is produced in the rat by chlorpromazine and reserpine (101) and by meprobamate (102, 103) and in the monkey by reserpine (104). In the rat, LTH is secreted by pituitary autografts regardless of the time of the cycle when the pituitary is transplanted or of the site in which it is placed (105).

Ergotoxine has no effect on FSH or LH content of pituitaries of pseudo-pregnant rats (106). In birds, prolactin temporarily depresses secretion of gonadotrophin (107).

The inhibitory effect of estrogens on FSH secretion seems to be mediated by the hypothalamus (108), and the secretion of LH can be impaired by bilateral lesions, ventral to the ventromedial nucleus of the hypothalamus (109). It has been confirmed that normal gonadotrophic function is dependent upon a direct anatomic contact between the pituitary and the median eminence of the hypothalamus (110, 111). In cats, stimulation of the amygdaloid nucleus produces ovulation (33). In the field mouse, summer lighting induces the appearance of vesiculated gonadotrophs in the pars distalis (112). In the male rat, vitamin-E deficiency produces atrophy of the seminiferous tubules and consequent changes in the peripheral gonadotrophs of the anterior pituitary (113). The pituitary of the catfish has a hormone similar to the LH of mammals (114). One case of ovarian mesenchymoma secreting HCG (115) has been reported.

Effects.—The weaver finch feather response may be obtained with HCG, the doses required being much larger than when LH is used (116). HCG increases urinary excretion of 17 KS, androsterone, etiocholanone, and estrogens (of testicular origin) in the human male (117, 118, 119); in the rat it decreases the ascorbic acid of the ovary (120), and in the newborn guinea pig produces maturation of the ovarian follicles and hyperplasia of the interstitial cells of the testes (121). Prolactin prolongs pregnancy in the rat by extending functional activity of corpora lutea (122) and is effective in stimulating the ovary of pregnant albino rats to produce enough additional progesterone to prevent the abortive action of ergotoxine (123).

In the rat, previous treatment with testosterone reduces the FSH effect of adenohipophysis implantations (124). In hypophysectomized immature rats, diethylstilbestrol increases the ovarian response to pituitary gonadotrophins but not to HCG or PMS (125). Thyroidectomy increases the ovarian response to PMS (126). In immature rats FSH administered intravenously has an antagonist action on the ovarian stimulation produced by adult male rat anterior pituitary homogenate given subcutaneously (127). Acetylation of the pituitary preparation inhibits its thyrothrophic effects but not its gonadotrophic activity (128).

Urinary excretion.—According to Loraine (129) HCG is the only hormone from the anterior pituitary and placenta which can be estimated with reasonable accuracy in both blood and urine. Measurements of urinary excretion of total gonadotrophins and of ICSH (130, 131, 132) have been made in human males and females, at different ages and throughout the menstrual

cycle (133). Human urinary (134) gonadotrophins contain both FSH and LH. Postmenopausal urine contains more FSH (both relatively and absolutely) than urine from young and middle-aged males (135, 136). In precocious puberty, increased ICSH (but not FSH) excretion has been reported (137). Absence of urinary FSH has been reported in four cases of eunuchoidism in the human female (138). FSH (separated from HCG) has been obtained from the urine of pregnant women (139).

A certain incompatibility for gonadotrophins exists between different species (140), and an antigonadotrophic serum has been obtained (141). Qualitative differences have been found between avian and mammalian gonadotrophins (142). The response to gonadotrophins may vary according to the strain employed in the assay (143). New methods for the extraction (144, 145, 146) for a more rapid biological test (147) and for quantitative determination of HCG (148, 149) have been reported.

ESTROGENS

The blood and tissue levels of estrogens have been determined by chemical methods in late human pregnancy (150 to 153). The results are shown in Table II.

Secretion and excretion.—The average excretion of estrogens during the

TABLE II
ESTROGEN CONCENTRATION IN LATE PREGNANCY IN $\mu\text{G. PER } 100 \text{ ML.}$

	Estrone	Estradiol-17 β	Estriol	Reference
Maternal blood	2.65–10.30	1.25–2.93	4.28–17.50	Aitken & Preedy (150)
	1.50–4.60	0.70–1.40	5.20–8.00	Roy & Brown (151)
free	1.00–3.00	2.00–6.00	3.00–13.00	Diczfalusy (152)
conjugated	1.00–4.00	0.10–0.30	4.00–20.00	
Cord blood	1.02–1.3	0.51–0.85	38.0–70.0	Roy & Brown (151)
Cord blood	1.02*	0.43*	57.1*	Diczfalusy & Magnusson (153)
Amniotic fluid	0.33*	0.27*	77.9*	
Fetal liver	0.75*	1.47*	24.00*	

* Geometrical mean values.

two peaks of the menstrual cycle range from 200 to 300 $\mu\text{g}/24$ hr. of estrogens. During menstruation excretion falls to 80 μg . per 24 hr. During the 10th week of pregnancy, the excretion ranges between 1 and 2 μg . per 24 hr. and increases progressively until the last weeks of gestation when it reaches values ranging from 30 to 100 μg . per 24 hr. (154). The turnover time of estrogens in blood has been estimated to about 6 min. or less (155). The urinary excretion of estrogens of the normal postmenopausal woman is comparable to that of the resting phase of the menstrual cycle, and to that found in the urine of the adult man. It is not reduced by bilateral oophorectomy (156). It seems that the ovary produces small amounts of estrogen in the absence of the pituitary (157). In women, estrogen excretion continues after oophorectomy and adrenalectomy (158, 159). In pregnant women a decrease of estrogen excretion before premature labor (160) and before normal labor (161) has been reported.

The production of estrogen by adrenocortical carcinoma is stimulated by ACTH (162). The excretion of 17-ketosteroids and estrogens was found to be abnormally high in a girl with a feminizing adrenal tumor (163). Adrenocortical carcinoma transplanted from a man to the anterior chamber of the guinea-pig eye maintained its ability to secrete estrogens (164). By chemical methods estrone and estradiol have been isolated from the urine of adult boars (165) and pregnant cows (166).

Biosynthesis and metabolism.—Estrogens are formed from androgens. The pathway of biosynthesis includes a 19-hydroxylated and probably 1- or 2-hydroxylated intermediate. A second pathway may include two isoprenoid units plus an endogenous C_9 compound. A mechanism for this pathway is suggested which involves the biosynthesis of equilenin and equilin as intermediates in the formation of estrone and estradiol-17 β (167). In bovine species, the *in vivo* conversion of estradiol-17 β to estrone and estradiol-17 α (168) and of estrone to estradiol-17 α has been demonstrated (169). In humans estradiol-17 β is also converted into 2-methoxyestrone (170), and 16 α -hydroxyestrone is converted into estriol, but not into estrone or into estradiol (430). The activity of rabbit red cells estronase is increased by glucose-6-phosphate (171).

The estrogens are the steroids most strongly bound to plasma proteins (172, 173), and the presence of liver tissue is obligatory for the *in vitro*-binding of estrogens with proteins (174). The hepatic inactivation of estrogens is reduced by low protein diet and restored by ascorbic acid (175). According to Müller (176), in male patients with liver cirrhosis the urinary excretion of estrogens is considerably higher than in normal, whereas Cameron (429) finds normal values in patients with chronic liver damage.

Determination.—An evaluation of the chemical methods for estimation of estrogens in urine has been made by Brown *et al.* (177). Several improvements to this method have been proposed (178, 179, 180). A good correlation has been found between chemical and biological estimation of urinary estrogens (181). New methods for extraction (182), separation (183), purifica-

tion (184), and for measurement (185) of estrogens in urine have been reported.

The effects of esterification of estrogens on the duration of their action are reviewed by Junkmann (186). The estradiol-17 β -n-valerate, the estradiol-17-cyclopentyl propionate (187, 188) and the polyestradiol phosphate (189) have a prolonged estrogen action on menopausal women. Two new synthetic nonsteroid estrogens have been studied (190 to 194).

Uterine effects.—In the uteri of mice, rats, hamsters, and guinea pigs, estrogens increase water, protein, nucleoproteins, glycogen (197, 198, 199), β -glucuronidase (199), succinoxidase (198), isocitric dehydrogenase (202), latic dehydrogenase-DPNH oxydase system, and DPN-cytochrome-C reductase (203). In the human myometrium, estrogens increase the concentration of contractile proteins (201); and in the endometrium of castrate rats, estrogens decrease the basal phospholipide but increase the apical phospholipide and Golgi apparatus (200).

According to their uterine growth-promoting action, the six naturally-occurring estrogens can be listed in order of potency as follows: estradiol-17 β ; estrone; 16 α -hydroxyestrone; 16 β -hydroxyestrone; estriol, and 16 epi-estriol (204). Both estriol and 16 epi-estriol display moderating effects upon the uterine growth-promoting action of estriol-17 β and estrone (204, 205). Progesterone displays a slight uterine growth-promoting effect: at low doses it interferes and at large doses it synergizes with estradiol-17 β . None of the pregnanediols or the pregnanediones induce uterine growth (204) or interfere with estradiol-17 β (206). Testosterone (207) and all corticoids inhibit to a large degree the action of estradiol-17 β (207, 208, 209).

Vaginal effects.—In the spayed rats, there is only summation (and not inhibition) of effects between different estrogens when studied on the vaginal smear (210), and estriol is almost as potent as estradiol in causing the development of the stratified epithelium in the vagina and cervix (211). In the spayed ewe the maximal vaginal response to estrogen is obtained 48 hours after progesterone treatment (212). There is a good correlation between urinary estrogen and vaginal cytology in women (213).

Effects on the mammary gland.—High and well balanced dosages of estrone and progesterone are required for promoting the normal mammary development in spayed guinea pigs (214). Estrone produced alveolar development of the mammary gland of castrate male guinea pigs, but not in castrate and adrenalectomized animals (215). Different strains of castrate male mice show marked differences in the mammary response to estrone and progesterone (216).

Effects on endocrine glands.—In female rats, the thyroid stimulating effects of estrogen is more pronounced when the adrenals are removed (217). Treatment with α -estradiol benzoate produces no marked alterations in thyroid size or thyroid-serum radioiodine concentration ratio (T/S)/ratio in hypophysectomized female rats (218). A slow increase of lipides in the adrenal cortex occurs after estradiol benzoate treatment in intact female or male rats, and a continuous decrease in spayed female or male rats (219).

Estrogens reduce the adrenal response to ACTH in women and the spayed guinea pig (220). In women, after discontinuance of estrogen therapy, ovarian functions are enhanced (rebound phenomena) (195). Estrogen produces regression of blastomata developed in splenic ovarian grafts (196).

Miscellaneous effects.—Quantitative studies have been made on mating behavior of spayed female cats stimulated by treatment with estrogens (221). Estradiol induces connective tissue hyperplasia in the seminal vesicles of immature rats (222). The dorsocranial part of the canine prostate gland is relatively only slightly sensitive to estrogenic stimulation (223). Diethylstilbestrol increases BMR and the weight of the hypophysis, thyroid, and adrenals, reduces the body growth of male rats (224, 225) and the spontaneous hypertrophy of the prostate in the dog (226). Estrogens have marked metabolic (227), protective (228), and regenerative (229) effects on the avian liver. Estradiol implanted into spayed female albino rats delayed the initiation and passage of the second wave of hair growth (230, 231).

Estrogens increase serum lipides and lipoproteins in amenorrheic and postmenopausal women (232) and in normal and eunuchoid men (233). Estradiol has a protective effect against experimental atherosclerosis in chickens (234) and also in rats with the exception of the coronary and renal arteries (235). In castrate women the incidence of atherosclerosis is higher than normal (236). In female hypogonadism, estrogens improve the olfactory perception (237). Estrogen decreases alkaline phosphatase (238) and increases serum copper (239) in human subjects. Estrogen stimulates the reticuloendothelial system function in mice (240) and decreases the abnormally high blood pressure of spayed chickens (241). Several papers have been published about experimental carcinogenesis with estrogens (242 to 245).

PROGESTERONE

Blood and placenta.—In the vein draining the active ovary of the ewe, the mean concentration of progesterone (determined by chemical methods) reached 1.8 $\mu\text{g.}$ per ml. from the 7th to the 16th day of the estrus cycle and also about the 17th week of pregnancy (248). Progesterone was not detected either in the blood of the vein draining the pregnant horn (248) or in the placenta of the ewe (249).

Short (250) finds, in pregnant women, values ranging between 6 and 21 $\mu\text{g.}$ per 100 ml. of plasma (in peripheral blood), and no decline during late pregnancy or labor. In cord blood, progesterone levels are five to ten times higher than in maternal blood. In pregnant cows, the level ranged from 0.74 to 0.97 $\mu\text{g.}$ per 100 ml. of plasma throughout the 32nd to 256th day period, but thereafter a marked decrease was observed (251).

Short (249) considers that "wide species differences exist in the hormonal mechanisms responsible for the maintenance of pregnancy." In the mouse during pregnancy, the plasma levels of progestin as determined by the Hooker-Forbes biological method show two peaks about the 8th and 15th day when progestin level reaches values equivalent to 8 $\mu\text{g.}$ of proges-

terone per cc. of plasma (246). Progesterin is also found in mouse embryos, placenta, and amniotic fluid from the 13th to the 18th day of pregnancy (247). By chemical analysis progesterone has been detected in the human placenta (252), and in the mare, but not in the ewe, cow, goat, and sow (249).

Biosynthesis and metabolism.—Davis and Plotz (253, 254, 255) have studied the biosynthesis and metabolism of progesterone by the use of radioactive precursors such as C^{14} acetate and tritium cholesterol and the metabolic pathway of the hormone after the administration of C^{14} -labelled progesterone. In humans, they confirm the synthesis of progesterone from acetate and cholesterol in the corpus luteum and the placenta. The principal pathway in the metabolism of progesterone during pregnancy has been shown to be acetate \rightarrow cholesterol \rightarrow Δ^5 -pregnenolone \rightarrow progesterone \rightarrow pregnanediol (253, 254, 255). Δ^4 -3-Ketopregnene-20 α -ol and Δ^4 -3-ketopregnene-20 β -ol have been isolated from human ripe follicles, corpora lutea, placentae and fat tissue (256), and in the bovine ovary (257). Both compounds are metabolites of progesterone and have progestational activity. Neither progesterone nor the Δ^4 -3-ketopregnene-20-ol fraction could be found in either muscle or mucosa of the pregnant uterus (256). This fact does not support the assumption that progesterone diffuses from the placenta to the myometrium.

17 α -hydroxyprogesterone and Δ^4 -androstene-3, 17-dione have been identified in extracts of human follicles, obtained prior to ovulation, and from corpora lutea (258). The following pathway is postulated for estrogen biosynthesis in the ovary: progesterone \rightarrow 17 α -hydroxyprogesterone \rightarrow Δ^4 -androstene-3,17-dione \rightarrow estrogens (258).

The rate of production of endogenous progesterone in late pregnancy has been determined to be in the order of 250 mg. per day, i.e. 170 μ g. per min. (259). The blood level being 0.08 μ g. per ml. of blood, the turnover time has been estimated to be 3.3 min. (155). Endogenous progesterone is transformed into biologically inactive products at a very rapid rate, the liver being the main site of inactivation (155).

Exogenous progesterone dissolved in propylene glycol injected intravenously to rabbits at the dose of 12 mg. per kg. of body weight produced a maximum blood level of 33 μ g. of progesterone per cc. The blood level fell very rapidly, becoming undetectable 30 min. after the injection (260), the half life being very close to 5 min. (260). In women, progesterone injected intravenously also disappears very rapidly from the blood stream (253), its half life being shorter than 7 min. (155). C -14 progesterone injected intravenously into human subjects was excreted in the urine (50 per cent) and in the bile (30 per cent) (261).

After injecting labelled progesterone, radioactive cortisone and hydrocortisone have been recovered from the urine (254). The *in vitro* incubation of progesterone with human adrenal tissue gives rise to a greater quantity of corticosterone than cortisol (262). Progesterone derivatives with oxidation at C -21 and C -11 retain a certain amount of antiluteinizing potency in

guinea pigs. No such potency is retained by derivatives with oxidation at C-17 which therefore can be considered as a landmark on the way from luteoid to corticoid functions (263). These results can explain why, in castrated animals, adrenalectomy removes an antiluteinizing factor (264).

Urinary excretion of pregnanediol.—In normal children, urinary pregnanediol has values similar to those found in adult men and postmenopausal women (265). During the menstrual cycle a basal level of approximately 1.0 mg. per 24 hr. is excreted by the adrenals. Three patterns of excretion, presumably reflecting the growth of the corpus luteum, could be discerned (266). In normal pregnant women a decline of pregnanediol excretion before labor has been reported (267) and denied (161, 312). A reduction of pregnanediol excretion has been confirmed in toxemia but not in cases of abortion (267). Urinary pregnanediol is increased by surgical stress and by ACTH administration; these effects are prevented by adrenalectomy (268). Pregnanetriol excretion rises during the last three months of pregnancy and after delivery rapidly falls to nonpregnant levels (269). When pregnane-21-ol-3,20-dione-succinate sodium is administered to human subjects, the chief excretory product is a glucuronide possessing a free 21-hydroxy-group. Pregnanediol could be detected in traces only (270). Several papers have been published about methods for pregnanediol (271, 272, 273) and pregnanetriol (274) determinations.

Effects.—Large doses (100 to 400 mg. per day) of intravenously administered progesterone produce in menopausal women a definite decrease in gonadotrophin excretion (98). In adult rats spayed two weeks previously, the uterine epithelium reacted to the treatment for 14 days with only 150 μ g. of progesterone by a measurable increase in the cell height (275). The increase in rabbit endometrial carbonic anhydrase has been used for the bioassay of progesterone (276). The decidual tissue formation produced by progesterone in spayed mouse is inhibited by cortisone, hydrocortisone, and ACTH (277), and by pregnane-3 α ,20 β -diol and pregnane-3 β ,20 β -diol (206). *In vitro*, progesterone at the concentration of 4 μ g. per ml. has toxic effects on the mouse ovum (278). It has been claimed that the eosinopenia of the luteal phase of the cycle is a direct effect of progesterone (279).

Esterification and halogenation.—The progestational activity of 17 α -hydroxyprogesterone becomes stronger and longer acting through acetate and caproate esterification (186, 280, 281, 282). The acetate ester has a progestational activity which is at least twice that of 20-21-anhydro-17 β -hydroxyprogesterone (283). Pregnancy has been maintained until term by a single dose of 17 α -hydroxyprogesterone caproate in spayed rabbits (284). The antiestrogenic effects are not enhanced by esterification (285). 9 α - and 12 α -halogenation increased progestational activity (286, 287) which is reduced by C-21 chlorination (288). The antiluteinizing potency of 11 β -hydroxyprogesterone increases by 9 α -esterification with fluor (289).

19-nor-steroids.—The suppression of the methyl group in the C-19 position seems to increase biological activity of the steroids (290). Extensive

studies in women have confirmed the progestational and antiovarulatory activity of 17 α -methyl-, 17 α -ethyl-, 17 α -ethynyl-19-nortestosterone, 19-norprogesterone and 17 α -ethynyl-5(10)estraen-17 β -ol-3-one (280, 291 to 296). After discontinuance of the medication, normal reproductive physiology seems to be reestablished (297), and in a group of subjects with previous inexplicable failure of reproduction 13 per cent of pregnancies have been reported (291, 298).

On intrauterine administration, 17 α -ethynyl-17-hydroxy-5(10)estren-3-one proved inactive as a progestational agent in the rabbit. When given subcutaneously, some response was obtained. Orally it was 10 to 25 times as effective as progesterone administered by the same route (299). Both orally and parenterally it acted like an estrogen on the uterine growth-stimulating test (300). The antiestrogenic effects [antifibromatogenic, antihysterotrophic (301), and anticoltrophic (302)] of 17-ethyl-19-nortestosterone are stronger than those of testosterone propionate. It has also androgenlike metabolic effects in humans (303, 304) and rats (305, 306). The effects of 17 α -ethyl- and 17 α -methyl-19-nortestosterone on the adrenal glands (307, 308), on the liver (309, 310), and on endometrial carbonic anhydrase (311) have been reported.

ANDROGENS

Biosynthesis.—Gonadal androgens and some of the adrenal androgens are produced by a pathway involving cholesterol, pregnenolone, progesterone, 17 α -progesterone, Δ 4-androstene-3,17-dione, and testosterone. A second pathway which is unique to the adrenal includes cholesterol, dehydroepiandrosterone, Δ 4-androstene-3,17-dione, 11 β -hydroxy- Δ 4-androstene-3,17-dione, and adrenosterone. Androgens are also formed by the action of peripheral tissue enzymes on corticoids possessing 17 α -hydroxy groups such as cortisol and 11-deoxycortisol (167, 313).

Blood levels.—The levels of testosterone and 4-androstene-3,17-dione in the spermatoc venous blood of dogs markedly increased within 30 min. after the injection of HCG (314). In 24 adult human males, the average plasma levels of dehydroepiandrosterone and of androsterone were respectively 48 and 25 μ g. per 100 ml. Adult females had slightly lower values. After the age of 40 years they decreased rapidly and were practically nil at 70 years (315). In intact and adrenalectomized male mice the administration of ACTH increases the plasma 17-ketosteroids. Adrenalectomized female mice have no significant plasma 17-ketosteroid levels (316).

Determination.—A modification for the biological assay of androgens by direct application to the combs of baby chicks (317) and several methods for determination of 17-ketosteroids and 17-ketogenic steroids (KGC) in human urine (318 to 324) and in cattle urine (325) have been published.

Effects.—Testosterone increases blood flow and diminishes the contractions of the seminal vesicles of adult rats which had been neonatally castrated (326); it increases the nitrogen retention of castrated male rats even

when the diet contains zein or gelatine as the only nitrogen sources (327). In women, androgens increase serum concentrations of α -2-globulin complex (328) and decreases serum α -lipoproteins, phospholipides, and cholesterol without changing their relative proportions (329). Testosterone does not correct the muscular atrophy in castrated and thyroidectomized guinea pigs (330); in normal women and children it reduces the plasma protein-bound iodine (PBI) concentration and iodine urinary excretion (331). In castrated male rats, testosterone inhibits the liver succinic dehydrogenase; it decreases adrenal weight and increases adrenal succinoxidase activity, thus reversing the alterations induced by castration (332, 333, 334).

Nor-androstenolonephenyl-propionate inhibits the atrophy of the adrenal cortex produced by cortisone in young male rats (335). A daily dose of 4 mg. produces slight hypertrophy of the adrenals but a daily dose of 0.2 mg. causes an additional increase in body weight and moderate atrophy of the adrenals (336). Testosterone corrects the atrophy of the adrenal cortex in hypothyroidic rats (337). Testosterone has no effect on the cells with metachromatic granulations of the cock's comb (338) and did not correct the effects of castration on sebaceous glands and the epidermis in hypophysectomized rats (339).

The effects of several esters of testosterone on the seminal vesicle weight of the castrated male rat increase in proportion to the length of the sterifying acid up to the enanthate. Esterification of androgens, however, does not always prolong and augment their effectiveness (186). The androgenic, anabolic, myotrophic, antiestrogenic, antigonadotrophic, and antifibromatogenic effects of testosterone have been compared with those of several derivatives including 19-nortestosterone (301, 304, 305, 340 to 344). A new synthetic phenentrene has a marked antiandrogenic activity (345). In rats, male plasma fibrin is found to be statistically higher in males than in females (346).

OVARY

In the human fetal ovary, extensive formation of primordial follicles occurs during the second half of pregnancy. During the 8th and 9th month, growth and atresia of follicles and differentiation of the cells of the theca interna also occur (347). The mechanical stimulation of the uteri, cervix, and vagina produce a marked reflex vasodilation of the ovarian vessels (348).

The proteins of the follicular fluid are similar to those of the blood plasma (350). Incorporation of radioactive sulfate sulfur in the follicular metachromatic sulfate mucopolysaccharide is marked and moves to the follicular fluid from the granulosa cells. This has been demonstrated by autoradiography in the rabbit ovary (351). An increase in the colloid osmotic pressure resulting from the enzymatic (352) degradation of the mucopolysaccharides present in the follicular fluid plays an important role in the ovulatory mechanism (353). A preovulatory increase occurs in the permeability of the blood liquor-barrier in mated rabbits (354). During the ovulatory phase of the menstrual

cycle in women (355), marked changes occur in the abdominovaginal electric potential, and the concentration of glucose on the cervical mucus reaches maximum values (356). The tests for ovulation in women have been extensively reviewed (357, 358). Ovulation has been produced in the cat by electrical stimulation of the amygdaloid nucleus (33) and is claimed to occur in humans after a single dose of conjugated equine estrogens (359, 360). Multiple ovulations have been induced in immature and adult rhesus monkeys with FSH (361) and in mature rats with PMS and HCG (362). Cortisone facilitates ovulation in the frog (364). Precocious ovulation has been observed in female pseudohermaphroditism (363).

In the living human ovum a tubular process which extends from each corona radiata cell through the zona pellucida enables a granular liquid to be emptied into the perivitelline space surrounding the egg (365). X-ray irradiation of the ovary reduces the number of oocytes and the fertility in rats (366) and mice (367). In the absence of the pituitary, the x-ray-sterilized ovary can produce more estrogen than a nonirradiated ovary (368). The rat ovary, in its normal site or implanted in a male which was castrated at birth, produces considerable amounts of androgen if placed under the influence of the high gonadotrophin levels (369). With the combined intrasplenic autotransplantation of the ovary and uterus in castrated rats, a marked hysterotrophic effect of the ovary can be observed whatever the distance between the grafts within the splenic pulp (349). The uterus left *in situ* however becomes atrophic. Normal saline is reported to be the best medium for treating ovarian grafts in the rat (370).

Relaxin.—There are marked contradictions among different authors. Whereas Birnberg (371), Stone *et al.* (372), Eichner *et al.* (373) claim that in pregnant women relaxin softens the uterine cervix and facilitates the progress of labor, Decker *et al.* (377) deny this effect. In rats a relaxing effect on the cervix has been reported (375). The inhibitory effect on uterine contractility has been confirmed in pregnant women (373) and applied to the treatment of premature labor (373, 376); this effect has been denied (377, 378) and relaxin considered inefficient in the treatment of premature labor (372, 377).

In the nonpregnant human uterus, relaxin has been shown to possess strong motility-inhibitory properties during the proliferative phase and less effectivity during the secretory phase (379). In dysmenorrheic patients, it has been reported to inhibit uterine contractions (379) but to be inefficient in the control of dysmenorrheic pain (380). Relaxin has no demonstrable effect on the spontaneous contractility of isolated human uterine muscle or on the tension of cervical tissues *in vitro*, taken from both nongravid and gravid uteri (374). In rats, relaxin [acting synergistically with estradiol-17 β (381, 382)] causes a marked increase of glycogen concentration preceded by an increase in uterine weight, water content, dry weight, and total nitrogen (383). The development of refractoriness to relaxin in guinea pigs has been attributed to the formation of an antihormone (384). In the rabbit, relaxin was observed to potentiate the progestational action of progesterone (382).

FEMALE GENITAL TRACT

Some of the mesenchymal portions of the uterus and tubes are formed by the mullerian and peritoneal linings after their initial differentiation into epithelium (392).

Fallopian tube.—Two different types of cells have been demonstrated by histochemical methods (385) and by tissue culture (386) in the tubal epithelium of rodents. In the rabbit, the ciliary activity of the fallopian tube gradually increases after copulation (387). Longitudinal contractions of the rabbit oviduct do not change in amplitude or rate at different intervals after mating up to four days, but they become greater in pregnancy and less in pseudopregnancy (388). The existence of sphincter mechanisms at the uterotubal orifices (389) has been deduced from transuterine insufflation in women. In the rabbit, the tubal contractions seem to be responsible for the fluctuations in the gas pressure which occur during uterotubal insufflation (390, 391).

Endometrium.—In the human endometrium, the sodium chloride and potassium content increases during the proliferative phase of the menstrual cycle. No variations in water content are noticed (394).

In the uterus and vagina of the rat, the highest activity of the DPN-diaphorase system and succinic dehydrogenase is associated with periods of maximal estrogen secretion (395). In women and monkeys, the reduction in endometrial thickness after the initial menstrual sloughing is caused primarily by loss of ground substance from the stroma. Secretory activity was also demonstrated in the preovulatory period (396). An increase of the metachromatic granules (heparinlike substance) has been found in the endometrial mast cell of pregnant and hypermenorrheic women (397). The protein content of the glandular epithelial nuclei has been studied with the interference contrast microscope (398). The biological activities of the endometrial secretions accumulated in experimental hydro-uteri of mice after ligation of their cervixes (399), and the endocrine factors which influence the rate of secretion (400) have been studied. Cyclic variations have been reported in the composition of the luminal fluids in bovine female genitalia (401).

Myometrium.—In the human nongravid myometrium, calcium and magnesium salts are found along the longitudinal axis of the ultramyofibrils at regular intervals. In the gravid myometrium, the structural properties as well as the thickness and period of the ultramyofibrils are similar to those found in the nongravid uterus (402). In the human myometrium the glycogen content (403) diminishes during menstruation, increases very much during pregnancy—as also happens in the rat (404)—and declines after menopause. Similar changes occur in the concentration of contractile proteins (201), ATP and ADP (405), and creatin phosphate (406). In the rabbit the administration of an aqueous extract of human myometrium prevents the posthysterectomy changes of the ovaries (407).

Muscle strips from the human nonpregnant uterus show that in the corpus, sinusoidal contractions are most common whereas in the cervix the

spontaneous motility is poor. Sphincterlike contractions are only found in the isthmus (408). The same type of spontaneous motility and pharmacological responses was observed in strips cut from uterine fibroids and in those obtained from normal myometrium (411). The inhalation anesthetics (with the exception of trichloroethylene) were found to be markedly depressant to gravid and nongravid uterine muscle activity *in vitro* whereas morphine, meperidine hydrochloride (Demerol), scopolamine, atropine, and phenobarbital produced no significant effects (412). By radiological methods, a sphincter mechanism has been found in the isthmus of the human nonpregnant uterus. This sphincter is most contractile in the luteal phase of the menstrual cycle and most relaxed before and during menstruation (409). Habitual abortion in the second trimester of pregnancy will be caused by traumatic or dysfunctional incompetence of the isthmus (410).

In the nongravid human uterus, *in situ* electrical activity is highest just prior to and immediately following menstruation (413). In the excised nongravid uterus no evidence of pacemaker activity was found (414). The action potentials of the rat and cat nonpregnant uterus have several features comparable to those of striated muscle (415). Estrogens increase the electrical activity of these uteri (416). The efficiency of myometrial circulation in removing radioactive Na^{22} injected into the thickness of the uterine wall has been studied in the nonpregnant woman, the average normal half-time found being four minutes (393).

Cervix.—The basic pattern of the epithelium of the cervix uteri in humans is formed by multiple grooves and innumerable finer irregular clefts arising from them (417). The cervical glands are able to concentrate I^{131} , S^{35} , and seroalbumin (418).

Sex cycles.—During normal menstrual cycle in women, crystallization of the saliva occurs during the estrogenic phase (420) but no evidence of cyclic changes of sodium or water balance was found (421, 422). Serum cholesterol increases during the follicular phase of the estrus cycle of albino rats (423). In normal women cyclic variations have been described in blood eosinophils (424), platelets (425), and in serotonin catabolite urinary excretion (640). Artificial vaginal cycles in spayed rats are inhibited by cortisone, prednisone, and hydrocortisone (426). Marked biochemical differences exist between menstrual blood and peripheral venous blood (419). Several reviews have been published on the metabolic, endocrine, and tissue changes which occur during artificial or spontaneous menopause in women (156, 236, 427). The cystic glands of postmenopausal human endometrium have no communication with any duct or with any other glandular structure (428).

PREGNANCY

Implantation.—The importance of histamine and the influence of antihistaminics, epinephrine, and ergotoxine in the experimental decidualoma have been studied in rats in an effort to elucidate the mechanisms of ova-implantation (431). In the rabbit three mechanisms operate during implanta-

tion: contractions of the myometrium which scatter the blastocyst; adhesion which anchors the blastocyst; and invasion by which the trophoblast penetrates the epithelium by displacement rather than destruction (432). In the guinea pig, the attachment cone of the blastocyst has been studied with time-lapse cinematography (433). Ovum transfer in the sheep was most successful when estrus was synchronized between donor and recipient. No two-cell ova and 15.8 per cent of four-cell ova developed when transferred into the uterine cornua (434).

In rabbits, postcoital ligation of the uterine tubes causes degeneration of the eggs; postcoital ovariectomy causes arrest of egg development up to 84 hours after mating. This can be prevented by progesterone only after the eggs have reached the uterus (435). In the rat, implantation is prevented by B-Z-55 (436) and the eggs are selectively destroyed by furazolidone (437) and by 1-phenyl-2p-anisylethanol (438) which do not affect the ovary or the genital tract.

A test for pregnancy based on the colour reaction of iodine with urine is described (439). In 56 per cent of cases of ectopic human pregnancy, characteristic endometrial changes were found (440).

Placenta.—Several good reviews (441, 442, 443) have been published on the placenta. Localization of homologous plasma proteins in normal and abnormal human placentas, by the fluorescent antibody technique, have shown proteins widely dispersed throughout the stromal ground substance of the villi, decidua, and cord, as well as in the intervillous spaces (444). The establishment of fetal-maternal vascular relations have been extensively studied by Patten (445), who finds that the chorionic villi at term are not in open pools, but are bathed in fluid seeping through a loosely organized substratum. A tentative classification of transport mechanisms across the human placenta has been proposed (446). In the human pregnancy at term, the surface of placental transfer has been estimated to be over 14 sq. m. and both the maternal and fetal placental blood flow at a rate of 500 cc. per min. (447, 448).

In human pregnancy, the comparison of antipyrine concentration in the umbilical vein with the calculated simultaneous intervillous concentration indicates that, at the time maternal and fetal blood are exposed at the placental barrier, equilibration of antipyrine is complete. Similar studies employing Na^{24} indicate that the diffusion of charged particles is impeded (449). In rabbits and cats, the distribution of P^{32} in the maternal and fetal organisms 30 minutes after intravenous injection at various stages of pregnancy showed that the concentration of the isotope was comparatively lower in the blood and almost all the organs of the fetus, with the exception of its musculature (450). The disaccharides were almost completely blocked by the rabbit placenta. Among the hexoses, and possibly among the pentoses, there was a clear differential permeability to the aldoses on one hand and the ketoses on the other (451).

In the rat, corticotrophin, thyroxine, triiodotironin, and insulin pass

through the placenta to the fetus, but the thyrotrophic hormone does not (452). In the rabbit insulin cannot pass from the fetus to the mother (453). In humans, cortisol can cross the "placental barrier" but in definite maternal-cord plasma ratio (454). In humans the placental transmission of poliomyelitis antibodies (455) and of the virus of cytomegalic inclusion body disease (456) has been demonstrated.

In normal human placentas, the concentration of HCG varied between 573 and 18 I.U. per gm. wet tissue, depending on the gestational age. A revised normal curve of placental HCG throughout pregnancy has been constructed (457). In the human placenta at term, 234 mg. of estradiol equivalents are found per kg. of wet tissue (458), and an epiestriol-like Kober chromogen (459) and a corticotrophic factor (460) have been isolated. The synthesis of free 17-hydroxycorticosteroids by the human placenta has been reported (461). The urinary excretion of gonadotrophins and estrogens has been used as a test for placental function (462).

The accelerated aging of placenta in toxemia of pregnancy produces an interference with simple metabolic and gaseous exchange in the later weeks of gestation, and a premature syncytial atrophy which is accompanied by a reduction in syncytial lipoids (463) and by lowered values for pregnanediol excretion (267).

Amnion.—The secretory activity of the amniotic cells in the placental area is suggested by their microscopic anatomy (464). Mitotic division of amniotic cells may be seen in early gestation but not in full term amnia (465, 466). In birds and reptiles, the amnion has a network of smooth muscle which exhibits rhythmic contractions (467).

Fetal fluids.—The physiology of fetal fluids (468) has received much attention. The origin and circulation of the amniotic fluid has been studied in humans and monkeys (469); and its composition has been studied in rats (470, 471, 472), humans (473), and sheep (474). The uptake of materials by the yolk sac has been studied in the rabbit (475).

Maternal changes during pregnancy.—There is an increase in the average body weight of women between the time of conception and two years after delivery (505), this increase being more significant for the short and thin women (506). The increase of the extrauterine weight of pregnant mice has been attributed to the ovarian progesterone (507, 508, 509). In the rat the growth-promoting activity of the plasma is increased during pregnancy (510).

In women the sodium space and the total exchangeable sodium are both increased during pregnancy (511). Similar increase has been reported for the water content of tissues and body fluids (512) whereas a decrease of total electrolytes, predominantly resulting from less serum sodium, has been found (513). The glomerular filtration rate, the renal plasma flow, and the creatinine clearance increase during the first trimester of pregnancy and then decrease until term, reaching values below the average levels of the normal nonpregnant subject. The filtration fraction is reduced during the first two trimesters of pregnancy but increases during the last trimester (514). Dis-

tion of the uterus of conscious dogs did not produce changes in the composition of the urine (515).

The total serum proteins and albumin fall during human gestation (512, 516, 517) whereas the α_1 -, α_2 - and β -globulin fractions steadily increase (517). Treatment with estrogens and progesterone did not produce changes in the hypoaminoacidemia of human pregnancy (517). The urinary excretion of a catabolite of serotonin increases during pregnancy (518). Adequate stores of glycogen are present in the maternal rat liver. The values obtained were slightly but significantly greater than nonpregnancy control values (519). In women glycogen mobilization by glucagon is unaffected by pregnancy (520), and glucose tolerance diminishes with increased parity (521), being independent of the plasma level of 17-OH-CS (524). In pregnant sheep, inanition induces a rapid depletion of carbohydrates and the accumulation of large amounts of fats in the liver (522). In the albino rat, pregnancy can afford protection against experimental calcinosis produced by dihydratachysterol (523). In women, the gastric functions are reduced during pregnancy (525, 526).

During normal human pregnancy, a decrease in hemoglobin content has been found (512). A hemoglobin level less than 12 gm. per 100 ml. in pregnant women should be considered as abnormal (527). The anemia of late pregnancy in the rabbit appears to be caused by a hemodilution of blood. A similar anemia was induced by treatment with estradiol (528). In human pregnancy, no correlation was found between the hemoglobin and B_{12} or folic acid blood levels (529). In late pregnancy, a decrease occurs in B_{12} blood level; in the fetus delivered at term, B_{12} levels are higher than in the mother (530). Eosinopenia occurs early in human pregnancy and an abrupt decrease of eosinophils is noticeable on the day preceding delivery (531). Fibrin, prothrombin, and platelets decrease during term pregnancy and increase during labor and the puerperium (532).

During human pregnancy, the thyroid has large follicles with abundant colloid and definite signs of cellular hypertrophy (533). Protein-bound iodine (PBI) and thyroxin in the serum of pregnant women showed a trend toward high values (533). The administration of triiodothyronine in the second and third trimester of pregnancy produces a depression of the elevated PBI level (534). In the rat, gestation increases renal excretion of I^{131} and simultaneously diminishes thyroïdal accumulation of the isotope (535). In the guinea pig, respiratory metabolism increases during pregnancy; thyroidectomy diminishes and thyroxin increases this phenomenon (536). In pregnant gilts, thiouracil produced a highly significant embryonic mortality, which was prevented by thyroprotein treatment (537).

In human pregnancy, there is an increase of the blood level (538) and the urinary excretion (538, 539, 540) of the 17-OH-CS, and in the total amount of the 17-KS and 17-KGS (538, 539). The absence of urinary aldosterone in the pregnant woman with Addison's disease (541, 542) suggests that aldosterone is not produced by the human placenta (541). In two cases of total adrenalectomy, subsequent normal pregnancies were obtained with

proper steroid-substitution therapy (543). A substance causing a distinct melanophore-dispersing action (544) has been obtained from urine of pregnant women.

Uterine contractility.—In the rabbit, until the 29th day of pregnancy, the myometrium is dominated by progesterone (79, 545) showing little spontaneous activity and low response to oxytocin. After the 29th day, a hormonal change occurs and during labor (32nd day) the myometrium becomes estrogen-dominated, with increased spontaneous contractility (545) and high response to oxytocin (79). After the 30th day 100 mU of oxytocin are able to induce labor (9), the threshold dose being 24 mU at term (79). With toxic doses (2000 to 8000 mU) of alpha-hypophamine (Pitocin), placental hemorrhages are produced (546) which were attributed to the vascular effects of vasopressin contaminating alpha-hypophamine since pure oxytocin at the dose of 16,000 mU did not produce any placental hemorrhage. The mechanism of labor in the rabbit (without recording uterine contractions) has been studied (8, 9).

In the human being, uterine contractility has been extensively studied during pregnancy and labor, recording the amniotic fluid pressure (40, 41, 60, 61, 73, 547, 548, 549) or the intrauterine pressures with carbon microphones (550) and with external pickups which are claimed to record the amniotic pressure (551, 552). Uterine activity is small until the 30th week of gestation and increases gradually during the last weeks of pregnancy (prelabor). As gestation approaches term, uterine activity increases more and more rapidly. There is no clear-cut demarcation between prelabor and labor. During labor, uterine activity increases until the fetus is delivered. The intensity of the contractions increases from 30 mm. Hg at the commencement of labor, to 50 mm. Hg at the end of labor. At the same time, the frequency increases from 3 to 5 contractions per 10 min., and the tonus from 8 to 12 mm. Hg. After the fetus is delivered, the uterus continues to produce rhythmic contractions (73), but uterine activity diminishes very rapidly (40, 41, 60, 61).

Overdistention of the uterus (such as occurs in polyhydramnios) raises the tonus and diminishes the amplitude of the contractions (553). The uterus shows great capacity of adaptation to the reduction of volume which occurs after the fetus and the placenta are delivered (554). The rupture of the fetal membranes diminishes the uterine work required for dilating the cervix (60, 61, 555) and also increases the intensity of the contractions (73) thus significantly shortening the duration of labor (556).

During labor, uterine contractions have marked hemodynamic consequences causing a rise of venous return, central venous pressure, cardiac output, arterial pressure (68, 73, 557), and spinal fluid pressure (558). Chlorpromazine has no effect on uterine contractility (72). In the human, progesterone neither prevents premature labor (559) nor reduces uterine response to oxytocin (74, 75). Using comparable doses of progesterone per kg. of body weight, there seems to be a difference in the uterine behavior of hu-

mans and rabbits (73). The above mentioned facts and the loss of myometrial potassium reported in the human during labor (560) do not support the hypothesis (80) that the withdrawal of progesterone and the consequent rise in myometrial intracellular potassium are the basic mechanisms responsible for the onset of labor in all species.

Electrohysterography in human pregnancy.—During labor, Larks *et al.* (561, 562), using DC amplifiers, recorded major biphasic slow waves, each corresponding to one uterine contraction, and considered this to reflect the spread of the excitation wave throughout the uterus. Evidence in favor of a pacemaker on the right uterine horn is presented. During pregnancy (563) the electrical activity is disorganized, increasing after the 35th week and becoming more coordinated as labor approaches. Larks' findings (561, 562, 563) confirm previously reported work from our laboratory on the origin, spreading, and coordination of the contractions of the pregnant human uterus. A much less clear picture is obtained with AC amplifiers (564). The potentials recorded from the skin may not accurately reflect uterine potentials (565). By stimulating the pregnant human uterus with exponential currents it has been possible to induce normal labor (566).

Circulation of the pregnant myometrium.—Effective blood circulation through the pregnant myometrium has been studied by the clearance rate of radiosodium injected in the uterine wall. The average time to half value ($T_{1/2}$) in normal pregnancies at term is around 5 min. When uterine contractility increases, as normally happens when labor advances, myometrial circulation is somewhat reduced (567). When uterine contractility becomes abnormally high and the $T_{1/2}$ is longer than 12 min., fetal distress appears. Marked reduction of myometrial flow occurs in toxemia of pregnancy and in pregnancy past term (568, 569). A reduction of uterine flow precedes premature labor (160).

Abortion and prolonged pregnancy.—The subject of abortion has been extensively reviewed (570, 571). Good results have been reported with antihistaminics (572) and with dibutoline sulfate and N-ethyl-3-piperidil diphenylacetate which are claimed to act as inhibitors of uterine contractility (573). With high dosages of progesterone, contradictory reports have been obtained (559, 574).

The physiology of "post term" or "post date" pregnancy has been extensively studied in humans with special attention directed to the placenta (575).

FETUS AND NEWBORN

Good discussions have been published on the aerobic and anaerobic metabolism of the human fetus (476), on the development of fetal immunity (477, 478), on the causes and prevention of fetal and neonatal distress (479), and on breathing and its control in premature infants (480).

Intrauterine color photography reveals no cyanosis of the human fetal skin and indicates a high oxygen saturation of the venous cord blood (493).

This saturation declines during the last weeks of pregnancy according to MacKay (481) whereas Rooth *et al.* (482) found constant levels from the 36th to the 45th week. The oxygen concentration of cord blood was reported to diminish in direct proportion to the age of the patient and the stage of pregnancy (484), whereas no correlation was found between these factors by Bancroft *et al.* (483). In the fetal lamb, only after the oxygen saturation falls to the level of 10 per cent is serious slowing of the heart encountered (487).

The fetal ECG has been recorded in humans as early as the 16th week of pregnancy (488). The heart rate of the human fetus has been recorded by several methods (66, 489, 490, 491). In the majority of cases of normal labor, the continuous ECG recordings did not reveal fetal bradycardia during uterine contractions (488). This bradycardia was recorded only in a minority of cases (492). Evidence of fetal respiratory movements during the second stage of labor has been reported in rabbits (485) where the prolongation of labor is marked by increase in the contamination of the air passages (486).

The sensitivity to epinephrine of the fetal rabbit cardiovascular system is similar to that of the adult animal (494). The plasma albumin fraction increases while the relative fetuin content (641) decreases with the advancing age of the fetal goat (495). In the sheep fetus, the rate of urine flow is maximal at 117 days of gestational age and then declines towards term (496). In humans, evidence of fetal micturition during labor has been obtained (497).

It has been claimed that chorionic gonadotrophin is responsible for the development and maintenance of the fetal zone of the adrenals in primates (498). 17-OH-CS in cord blood of the anencephalic human fetus at term is similar to that observed in normal infants suggesting that the fetal zone of the adrenal cortex is not responsible for their production (499). The 17-OH-CS level is significantly higher in venous than in arterial cord blood (500). Wide variations in epiphyseal ossification has been found in twins (502) and in fetuses of similar gestational age (501). In newborn infants, hypoxia significantly reduces heat production (503), and the respiratory minute volume is decreased by maternal analgesia with pethidine and increased by nikethamide (504).

LACTATION

Excellent reviews have appeared on mammary development and lactation (576) as well as on factors affecting the composition (577) and the rate of secretion of milk (578).

Cortisol acetate stimulated mammary development in ovariectomized estrogen-treated albino mice (579). In lactating goats there was a rapid inhibition of milk secretion after adrenalectomy. Deoxycorticosterone acetate was the most critical component for complete maintenance of lactation (580). A slight regression in adrenal cortical activity during the latter half of lactation was observed in rats (581). In the hypophysectomized rats, on the 4th day of lactation, ACTH enhanced the replacement value of prolactin (582).

In rabbits, the destruction of the hypothalamohypophysial portal vessels

reduces the growing rate of the litter, which is unaffected by lesions of the stem which do not destroy the portal vessels (583). In the lactating cows, there is a highly significant linear relationship between log weight of growth hormone injected and the increase in milk yield obtained (584). The reproductive tract of the lactating mouse shows progesterone effects until the 11th day, after which the effects become progressively more estrogenic (585). In the field mouse, lactation neither prevented the occurrence of estrus cycles nor changed the duration of pregnancy (586). Lactation in the woman was associated with a significant increase in gastric secretion which did not occur in patients who were not breast feeding (525). In humans, the Montgomery tubercles are capable of secreting milk (587).

MALE SEX ORGANS

Testis.—In the rabbit the hormone secreted by the fetal testis is different from that of the adult animal. The pituitary gonadotrophins are indispensable for testicular function only during a certain period of fetal life (588). The ultrastructure of the human spermatozoon (589), spermatocytes, and interstitial cells (590), and the vasa efferentia of the hamster (591) has been studied. A good review of the endocrine function of the testis has been presented by Benoit (592). The Leydig cells of normal adults have all the characteristics of endocrine cells (593). A cycle from birth to puberty is found in germinative and interstitial cells (594, 595). The delayed production of a blastomatous formation occurs after ligation of testicular vessels (596). A great proliferation occurs in the Leydig cells of testis graft in the spleen of the castrated rat (597). Hyaluronidase concentration paralleled that of the spermatids in the testicular tubules of rats treated with Fura-droxyl (5-nitro-2-furaldehyde 2-(2-hydroxymethyl) semicarbazone) or x-rays (598). A second testicular hormone of estrogenic nature is claimed by Forbes (599). In the guinea pig, endocrine activity of the testis was reduced after three years of experimental cryptorchidism, but the reduction was not sufficient to interfere with maintenance of secondary sex characteristics (600).

Spermatogenesis.—In rats, ablation of the anterior areas of the brain cortex produces testicular atrophy, involution of the genital tract, and loss of libido (601). In the guinea pig, the destruction of the prostate-vesicular sympathetic ganglion produces azoospermia and testicular atrophy (602). In hypophysectomized rats, pituitary gonadotrophins are indispensable for normal spermatogenesis and cannot be replaced by HCG or by PMS (603). In *Rana pipiens*, FSH stimulates spermatogenic proliferation and LH induces spermatogenesis and interstitial cell stimulation (604, 605). In *Bufo arenarum* (H) spermatogenesis is continuous and HCG produces a rapid increase lasting for 30 to 90 minutes (606).

In the human it has been claimed that the administration of vitamin E to the male prior to conception reduces the rate of fetal abnormalities (607). Testicular biopsies show a quantitative relationship between the composition

of the sperm and the histologic picture of the seminiferous tubules (608). In rats, tolbutamide or insulin produces lesions of the germinal epithelium which can be prevented by simultaneous administration of glucose (609).

Sperm.—The low content of deoxyribose nucleic acid in sperm may be a possible cause of human infertility (610). The rate of fructolysis of human semen is used as a test of metabolic activity of spermatozoa (611). Bull spermatozoa metabolize glycerol and sorbitol, producing lactic acid (612); previous treatment with glycerol increases their resistance to low temperature (613). Ethylenediamine acetic acid inhibits motility and anaerobic fructolysis of human sperm (614). Absence of fructose and citric acid is found in semen of bulls with vitamin-B deficiency; this defect is corrected by androgens or gonadotrophins (615). In cases of human oligospermia, globulins appear in the sperm (616).

Genital tract.—Electrical stimulation of the dorsal lateral or posterior areas of the hypothalamus in adult male rabbits evoked an immediate contraction of the seminal vesicle followed by delayed contractile effect. Contractions of the cauda epididymis and vas deferens also occurred. Section of the hypogastric nerves abolishes the immediate response, and adrenalectomy the delayed effect. Stimulation of the peripheral end of the cut hypogastric nerves or injection of 1 to 5 μ g. epinephrine causes the two types of response. Neither electrical stimulation of the neurohypophysis nor injection of oxytocin or vasopressin induces a contraction of the seminal vesicle (617). In paraplegic and quadriplegic human males, the lower the section of the spinal cord involved, the greater the disturbances in erection and ejaculation (618).

SEX DETERMINATION AND DIFFERENTIATION

Few new experimental facts have been established, but a considerable number of interpretations on the development of the gonads and the secondary sexual characters have been published (619 to 622, 639). The sex chromatin mass has received much attention (623 to 630) and the test for this mass, together with Jost's data, has facilitated a better understanding of gonadal dysgenesis.

Based on the study of Turner's syndrome with male chromatin sex (619, 620, 631, 632, 633), true Klinefelter's syndrome with female chromatin sex (619, 621, 632), and male pseudohermaphrodism (619, 620, 632, 634, 635), the following has been postulated: the existence of fetal "male organizing hormone" (632) produced by the medullary component of the fetal gonad; the fact that the maternal organism responds to the differentiation of the fetal testes by the production of an "antagonist" which suppresses the male induction and endocrine capacities of the testes (619, 620).

Android changes in the external genitalia of female babies born of mothers treated with large doses of progesterone (622), 17-ethynil-testosterone (636), and androgens (632) have been observed.

Immersion of fertile hen's eggs into solutions of estrogens produces

feminization of the genetic male chicks (637). Cephalic integrity or hypothalamic control is not essential for differentiation of genetically determined sex in man (638).

It is apparent that research on the effect of hormones on reproduction continues apace at an increasing rate. While new problems continually become apparent and many old problems remain unsolved, some of the controversies are resolved; and our understanding of the subject continues to increase in spite of the complexity of this important field of research

LITERATURE CITED

1. Bisset, G. W., and Lee, J., *Lancet*, **I**, 1173-74 (1957)
2. Bisset, G. W., Lee, J., and Bromwich, A. F., *Lancet*, **II**, 1129-32 (1956)
3. Heller, H., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 3-14 (1957)
4. Semm, K., *Der Oxytocin-Oxytocinase-Haushalt unter besonderer Berücksichtigung des Wehen-Problems* (Doctoral thesis, Ludwig-Maximilians-Universität München, Munich, Germany, 93 pp., 1958)
5. Fitzpatrick, R. J., *The Neurohypophysis*, 203-17 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
6. Cross, B. A., *J. Endocrinol.*, **16**, 237-60 (1958)
7. Berde, B., Doepfner W., and Konzett, H., *Brit. J. Pharmacol.*, **12**, 209-14 (1957)
8. Berde, B., and Cerletti, A., *Acta Endocrinol.*, **27**, 314-24 (1958)
9. Cross, B. A., *J. Endocrinol.*, **16**, 261-76 (1958)
10. Méndez-Bauer, C., Carballo, M. A., and Pose, S. V., *Proc. 3rd Latin-Am. Congr. Obstet. Gynecol.*, **2**, 24-26 (1958)
11. Méndez-Bauer, C., and Carballo, M. A., *Proc. 4th Panam. Congr. Endocrinol.*, 293-94 (1957)
12. Boissonnas, R. A., Guttman, S., Jaquenoud, P. A., and Waller, J. P., *Helv. Chim. Acta*, **39**, 1421 (1956)
13. Boissonnas, R. A., Guttman S., Jaquenoud, P. A., Waller, J. P., Konzett, H., and Berde, B., *Nature*, **178**, 260 (1956)
14. Berde, B., and Cerletti, A., *Gynaecologia*, **144**, 275-78 (1957)
15. Caldeyro-Barcia, R. (Unpublished observations, 1958)
16. Croxatto, H., *The Neurohypophysis*, 51-60 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
17. Hawker, R. W., and Robertson, P. A., *Nature*, **180**, 343-44 (1957)
18. Hawker, R. W., and Robertson, P. A., *Endocrinology*, **60**, 652-57 (1957)
19. Hawker, R. W., and Robertson, P. A., *J. Clin. Endocrinol. and Metabolism*, **17**, 448-51 (1957)
20. Acher, R., and Fromageot, C., *The Neurohypophysis*, 39-48 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
21. Van Dyke, H. B., Adamsons, K., Jr., and Engel Stanford, L., *The Neurohypophysis*, 65-73 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
22. Dale, H. H., *The Neurohypophysis*, 1-9 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
23. Hanström, B., *Nova Acta Regiae Soc. Sci. Upsaliensis*, **17**, 3-12 (1957)
24. Kivalo, E., and Talanti, S., *Acta Endocrinol.*, **26**, 128-34 (1957)
25. Bargmann, W., *The Neurohypophysis*, 11-18 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
26. Kivalo, E., and Talanti, S., *Acta Endocrinol.*, **26**, 471-76 (1957)
27. Lasansky, A., and Sabatini, D. D., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 110 (1957)
28. Hanström, B., *The Neurohypophysis*, 23-34 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
29. Tramezzani, H. J., Negreiros de Paiva, C., and Antúnez, C., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 173-74 (1957)
30. Ginsburg, M., and Brown, L. M., *The Neurohypophysis*, 109-30 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)

31. Croxatto, H., and Fernández, J., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 62-63 (1957)
32. Ampuero, O., and Croxatto, H., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 27-28 (1957)
33. Shealy, C. N., and Peele, T. L., *J. Neurophysiol.*, **20**, 125-39 (1957)
34. Cross, B. A., Goodwin, R. F. W., and Silver, I. A., *J. Endocrinol.*, **17**, 63-74 (1958)
35. Hawker, R. W., and Roberts, V. S., *Brit. Veterinary J.*, **113**, 459-64 (1957)
36. Hawker, R. W., *J. Clin. Endocrinol. and Metabolism*, **18**, 54-60 (1958)
37. Hendricks, C. H., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 99 (1957)
38. Walker, J. M., *The Neurohypophysis*, 221-29 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
39. Bisset, G. W., and Walker, J. M., *Brit. J. Pharmacol.*, **12**, 461-67 (1957)
40. Caldeyro-Barcia, R., *Proc. 4th Panam. Congr. Endocrinol.*, main lecture, 9-21 (1957)
41. Caldeyro-Barcia, R., and Poseiro, J. J., *Ann. N. Y. Acad. Sci.*, **75**, 813-30 (1958)
42. González-Panizza, V. H., *Proc. 4th Panam. Congr. Endocrinol.*, 257 (1957)
43. Reuse, J. J., and Vanden Driessche, R., *J. physiol. (Paris)*, **49**, 358-60 (1957)
44. Croxatto, H., *Proc. 4th Panam. Congr. Endocrinol.*, 33-35 (1957)
45. Hawker, R. W., *Quart. J. Exptl. Physiol.*, **41**, 301-8 (1956)
46. Semm, K., *Naturwissenschaften*, **44**, 424-25 (1957)
47. Méndez-Bauer, C., and Carballo, M. A., *Proc. 2nd Uruguayan Congr. Obstet. Gynecol.*, **2**, 31-35 (1957)
48. Carballo, M. A., *Ocitocinasa* (Doctoral thesis, Universidad Nacional de Córdoba, Córdoba, Argentina, 58 pp., 1958)
49. Dicker, S. E., and Tyler, C., *J. Obstet. Gynaecol. Brit. Empire*, **63**, 690-96 (1956)
50. Carballo, M. A., and Méndez-Bauer, C., *Proc. 2nd Uruguayan Congr. Obstet. Gynecol.*, **2**, 42-49 (1957)
51. Dicker, S. E. (Personal communication, 1958)
52. Samuels, L. T., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 17 (1957)
53. Sica-Blanco, Y., and González-Panizza, V. H., *Proc. 2nd Uruguayan Congr. Obstet. Gynecol.*, **2**, 117-23 (1957)
54. Sica-Blanco, Y., *Proc. 4th Panam. Congr. Endocrinol.*, 342-43 (1957)
55. Chaudhury, R. R., and Walker, J. M., *J. Physiol. (London)*, **138**, 50P-51P (1957)
56. Noble, R. L., *The Neurohypophysis*, 97-106 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
57. Heller, H., *The Neurohypophysis*, 77-94 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
58. Imanaga, H., Kondo, T., and Mori, I., *J. Clin. Endocrinol. and Metabolism*, **17**, 1081-87 (1957)
59. Dicker, S. E., and Greenbaum, A. L., *J. Physiol. (London)*, **141**, 107-16 (1958)
60. Caldeyro-Barcia, R., Alvarez, H., Poseiro, J. J., Hendricks, C. H., Sica-Blanco, Y., Pose, S. V., Cibils, L. A., Carballo, M. A., González-Panizza, V., Fielitz, C., Méndez-Bauer, C., and de Telias, E. A., *2nd Uruguayan Congr. Obstet. Gynecol.*, Main lectures, **1**, 5-88 (1957)
61. Caldeyro-Barcia, R., Alvarez, H., Poseiro, J. J., Pose, S. V., Cibils, L. A., Sica-Blanco, Y., Carballo, M. A., Méndez-Bauer, C., González-Panizza, V. H., and Fielitz, C., *3rd Latin-Am. Congr. Obstet. Gynecol.*, Main lectures, **1**, 131-65 (1958)

62. Caldeyro-Barcia, R., Sica-Blanco, Y., Poseiro, J. J., González-Panizza, V. H., Méndez-Bauer, C., Fielitz, C., Alvarez, H., Pose, S. V., and Hendricks, C. H., *J. Pharmacol. Exptl. Therap.*, **121**, 18-31 (1957)
63. Pose, S. V., *Excerpta Med.*, Sec. X, 37 (2nd World Congr. Intern. Fed. Gynecol. Obstet., free communication, 1958)
64. Aburel, E., Petrescu, V., and Radulescu, E., *Minerva med.*, **31**, 1365-70 (1957)
65. Smyth, C. N., *Lancet*, **I**, 237-39 (1958)
66. Poseiro, J. J., *Excerpta med.*, Sec. X, 38 (2nd World Congr. Intern. Fed. Gynecol. Obstet., free communication, 1958)
67. Cibils, L. A., and Caldeyro-Barcia, R., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 53 (1957)
68. Hendricks, C. H., *Proc. 4th Panam. Congr. Endocrinol.*, 262 (1957)
69. Pose, S. V., *Proc. 2nd Uruguayan Congr. Obstet. Gynecol.*, **2**, 129-36 (1957)
70. Alvarez, H., Caldeyro-Barcia, R., Cerviño, J. M., and Mussio-Fournier, J. C., *Proc. 4th Panam. Congr. Endocrinol.*, 191 (1957)
71. Poseiro, J. J., *Proc. 2nd Uruguayan Congr. Obstet. Gynecol.*, **2**, 102-7 (1957)
72. Caldeyro-Barcia, R., Poseiro, J. J., Alvarez, H., and Tost, P., *Am. J. Obstet. Gynecol.*, **75**, 1088-95 (1958)
73. Caldeyro-Barcia, R., *Excerpta Med.*, Sect. X, 4 (2nd World Congr. Intern. Fed. Gynecol. Obstet., main lecture, 1958)
74. Pose, S. V., Poseiro, J. J., and Fielitz, C. A., *Proc. 4th Panam. Congr. Endocrinol.*, 318 (1957)
75. Fielitz, C. A., and Caldeyro-Barcia, R., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 86 (1957)
76. Aburel, E., Petrescu, V., and Radulescu, E., *Obstet. si Ginecol.*, **5**, 217-26 (1957)
77. Petrescu, V., and Rubin, Z., *Obstet. si Ginecol.*, **4**, 328-34 (1956)
78. Petrescu, V., *Obstet. si Ginecol.*, **6**, 127-34 (1958)
79. Schofield, B. M., *J. Physiol. (London)*, **138**, 1-10 (1957)
80. Csapó, A., *Ann. N. Y. Acad. Sci.*, **75**, 790-808 (1958)
81. Hoff, F., and Bayer, R., *Z. Geburtshilfe u. Gynäkol.*, **148**, 233-70 (1957)
82. Steinetz, B. G., Beach, V. L., and Kroc, R. L., *Endocrinology*, **61**, 271-80 (1957)
83. Kroc, R. L., Steinetz, B. G., and Beach, V. L., *Ann. N. Y. Acad. Sci.*, **75**, 942-80 (1958)
84. Cowie, A. T., and Folley, S. J., *The Neurohypophysis*, 183-97 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
85. Benson, G. K., and Folley, S. J., *J. Endocrinol.*, **16**, 189-201 (1957)
86. González Cuesta, A., *El Médico*, **7**, 48-53 (1958)
87. Labarca, E., and Croxatto, H., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 109-10 (1957)
88. Croxatto, H., Rosas, R., and Barnafí, L., *Acta Physiol. Latinoam.*, **6**, 147-52 (1956)
89. Rosas, R., Croxatto, H., and Navarrete, E., *Proc. 4th Panam. Congr. Endocrinol.*, 330-31 (1957)
90. Lastra, M. de la, and Croxatto, H., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 110-11 (1957)
91. Berde, B., and Cerletti, A., *Helv. Physiol. et Pharmacol. Acta*, **14**, 129-34 (1956)
92. Croxatto, H., and Zamorano, B., *Acta Physiol. Latinoam.*, **7**, 33-38 (1957)
93. Brooks, F. P., and Pickford, M., *The Neurohypophysis*, 141-53 (Butterworths Scientific Publications, London, Engl., 275 pp., 1957)
94. Landon, D. R., and Claringbold, P. J., *J. Endocrinol.*, **16**, 298-303 (1958)

95. Aron, M., and Aron, C., *La fonction endocrine du testicule*, 70-108 (Masson et Cie, Paris, France, 532 pp., 1957)
96. Raacke, I. D., Lostroh, A. J., Boda, J. M., and Li, C. H., *Acta Endocrinol.*, **26**, 377-87 (1957)
97. Greenblatt, R. B., *Fertility and Sterility*, **8**, 537-46 (1957)
98. Rothchild, I., *J. Clin. Endocrinol. and Metabolism*, **17**, 754-59 (1957)
99. Chambon, M. Y., *Bull. fédération soc. gynécol. et obstét. langue franç.*, **9**, 337-38 (1957)
100. Velardo, J. T., *Fertility and Sterility*, **9**, 60-66 (1958)
101. Barraclough, C. A., and Sawyer, C. H., *Endocrinology*, **61**, 341-51 (1957)
102. Gitsch, E., and Everett, J. W., *Endocrinology*, **62**, 400-9 (1958)
103. Gitsch, E., *Endocrinology*, **62**, 533-35 (1958)
104. De Feo, V. J., and Reynolds, S. R. M., *Rev. ginecol. obstet. (Rio de Janeiro)*, **100**, 53-61 (1957)
105. Nikitovitch-Winer, M., and Everett, J. W., *Endocrinology*, **62**, 522-32 (1958)
106. Shelesnyak, M. C., *Endocrinology*, **60**, 802-3 (1957)
107. Lofts, B., and Marshall, A. J., *J. Endocrinol.*, **17**, 91-98 (1958)
108. Flerkó, B., and Szentágothai, J., *Acta Endocrinol.*, **26**, 121-27 (1957)
109. Van Dyke, D. C., Simpson, M. E., Lepkovsky, S., Koneff, A. A., and Brobeck, J. R., *Proc. Soc. Exptl. Biol. Med.*, **95**, 1-5 (1957)
110. Greer, M. A., *Recent Prog. in Hormone Research*, **13**, 67-98 (1957)
111. Iltf, J. D., *Endocrinology*, **61**, 595-96 (1957)
112. Clarke, J. R., *J. Endocrinol.*, **15**, liv (1957)
113. Griesbach, W. E., Bell, M. E., and Livingston, M., *Endocrinology*, **60**, 729-40 (1957)
114. Ramaswami, L. S., and Sundararaj, B. I., *Acta Endocrinol.*, **27**, 253-56 (1958)
115. Secher, E., *Acta Obstet. Gynecol. Scand.*, **37**, 97-101 (1958)
116. Segal, S. J., *Science*, **126**, 1242-43 (1957)
117. Jayle, M. F., Scholler, R., Garrone, G., and Morel, F., *La fonction endocrine du testicule*, 201-21 (Masson et Cie., Paris, France, 532 pp., 1957)
118. Huis in't Veld, L. G., *La fonction endocrine du testicule*, 139-56 (Masson et Cie., Paris, France, 532 pp., 1957)
119. Halkerston, I. D. K., Hillman, J., Palmer, D., Reiss, M., and Rundle, A., *J. Endocrinol.*, **16**, 156-63 (1957)
120. Noach, E. L., and van Rees, G. P., *Acta Endocrinol.*, **27**, 502-8 (1958)
121. Buño, W., *Proc. 4th Panam. Congr. Endocrinol.*, 209 (1957)
122. Meites, J., and Shelesnyak, M. C., *Proc. Soc. Exptl. Biol. Med.*, **94**, 746-49 (1957)
123. Shelesnyak, M. C., *Acta Endocrinol.*, **27**, 99-109 (1958)
124. Paesi, F. J. A., de Jongh, S. E., and Engelbregt, A., *Acta Endocrinol.*, **25**, 412-18 (1957)
125. Payne, R. W., and Runser, R. H., *Endocrinology*, **62**, 313-21 (1958)
126. Mandl, A. M., *J. Endocrinol.*, **15**, 448-57 (1957)
127. Talbert, G. B., Di Pillo, F., and Gordis, L., *Endocrinology*, **61**, 611-17 (1957)
128. Sonenberg, M., and Money, W. L., *Endocrinology*, **61**, 12-19 (1957)
129. Loraine, J. A., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 19-33 (1957)
130. McArthur, J. W., Ingersoll, F. M., and Worcester, J., *J. Clin. Endocrinol. and Metabolism*, **18**, 460-69 (1958)
131. Henry, R., *La fonction endocrine du testicule*, 179-200 (Masson et Cie., Paris, France, 532 pp., 1957)

132. Buchholz, R., *Geburtshilfe u. Frauenheilk.*, **17**, 707-16 (1957)
133. Borth, R., Lunenfeld, B., and de Watteville, H., *Fertility and Sterility*, **8**, 233-54 (1957)
134. Albert, A., Kelly, S., Silver, L., Kobi, J., and Bloodsworth, L., *J. Clin. Endocrinol. and Metabolism*, **18**, 453-59 (1958)
135. Hamburger, C., and Johnsen, S. G., *Acta Endocrinol.*, **26**, 1-29 (1957)
136. Walter, K., *J. Endocrinol.*, **15**, 119-25 (1957)
137. Morat6 Manaro, J., Cervi6o, J. M., Maggiolo, J., Navarro, A., and Pollak, E., *Rev. arg. endocrinol. metabolismo*, **4**, 1-13 (1958)
138. Moldawer, M. P., Albright, F., Benedict, P. H., Forbes, A. P., and Henneman, P. H., *J. Clin. Endocrinol. and Metabolism*, **18**, 1-14 (1958)
139. Butt, W. R., Crooke, A. C., Ingram, J. D., and Round, B. P., *J. Endocrinol.*, **16**, 107-13 (1957)
140. Nissim, J. A., *J. Physiol. (London)*, **140**, 13P-14P (1958)
141. Ely, C. A., *Endocrinology*, **60**, 718-28 (1957)
142. Taber, E., Claytor, M., Knight, J., Gambrell, D., Flowers, J., and Ayers, C., *Endocrinology*, **62**, 84-89 (1958)
143. Brown, P. S., *J. Clin. Endocrinol. and Metabolism*, **17**, 805-8 (1957)
144. Butt, W. R., *J. Endocrinol.*, **15**, lix (1957)
145. Butt, W. R., and Round, B. P., *J. Endocrinol.*, **17**, 75-80 (1958)
146. Albert, A., and Silver, L., *Endocrinology*, **61**, 587-90 (1957)
147. Frazer, J. F. D., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 883-84 (1957)
148. Moisset de Espanes, B., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 127-28 (1957)
149. Rosenberg, E., Smith, F., and Dorfman, R. I., *Endocrinology*, **61**, 337-40 (1957)
150. Aitken, E. H., and Preedy, J. R. K., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 331-34 (1957)
151. Roy, E., and Brown, J. B. (quoted by Loraine), *Ciba Foundation Colloq. on Endocrinol.*, **11**, 335 (1957)
152. Diczfalusy, E., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 336-37 (1957)
153. Diczfalusy, E., and Magnusson, A. M., *Acta Endocrinol.*, **28**, 169-85 (1958)
154. Brown, J. B., *J. Endocrinol.*, **16**, 202-12 (1957)
155. Pearlman, W. H., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 233-48 (1957)
156. McBride, J. M., *J. Clin. Endocrinol. and Metabolism*, **17**, 1440-47 (1957)
157. Ingram, D. L., and Mandl, A. M., *J. Endocrinol.*, **17**, 13-16 (1958)
158. Greenwood, F. C., and Bulbrook, R. D., *J. Physiol. (London)*, **137**, 39P (1957)
159. Bulbrook, R. D., and Greenwood, F. C., *Brit. Med. J.*, **I**, 622-68 (1957)
160. Bruns, P. D., Taylor, E. S., Anker, R. M., Drose, V. E., *Am. J. Obstet. Gynecol.*, **73**, 579-86 (1957)
161. Khakimova, S., *Some Peculiarities of Neuro-Hormonal Regulation of Uterine Contractility in Normal and Pathological Pregnancy* (Thesis, 1st Medical Institute, Moscow, U.S.S.R., 1957)
162. West, C. D., Damast, B., and Pearson, O. H., *J. Clin. Endocrinol. and Metabolism*, **18**, 15-27 (1958)
163. Snaith, A. H., *J. Clin. Endocrinol. and Metabolism*, **18**, 318-22 (1958)
164. Wolf, E. T., Mills, L. C., Newton, B. L., Tuttle, L. L. D., Hettig, R. A., Collins, V. P., and Gordon, W. B., *J. Clin. Endocrinol. and Metabolism*, **18**, 310-17 (1958)
165. Velle, W., *Acta Endocrinol.*, **28**, 255-61 (1958)
166. Velle, W., *Acta Endocrinol.*, **27**, 64-72 (1958)

167. Dorfman, R. I., *4th Panam. Congr. Endocrinol.*, main lecture, 51-68 (1957)
168. Velle, W., *Acta Endocrinol.*, **28**, 186-91 (1958)
169. Velle, W., *Acta Endocrinol.*, **28**, 192-96 (1958)
170. Engel, L. L., Baggett, B., and Carter, P., *Endocrinology*, **61**, 113-14 (1957)
171. Bischoff, F., Torres, A., and López, G., *Am. J. Physiol.*, **189**, 447-50 (1957)
172. Sandberg, A. A., Slaunwhite, W. R., Jr., and Antoniades, H. N., *Recent Progr. in Hormone Research*, **13**, 209-60 (1957)
173. Antoniades, H. N., McArthur, J. W., Pennell, R. B., Ingersoll, F. M., Ulfelder, H., and Oncley, J. L., *Am. J. Physiol.*, **189**, 455-59 (1957)
174. Szego, C. M., *Ciba Foundation Colloq. on Endocrinol.*, **II**, 286-305 (1957)
175. Vasington, F. D., Parker, A., Headley, W., and Vanderlinde, R. E., *Endocrinology*, **62**, 557-64 (1958)
176. Müller, J., *Acta Endocrinol.*, **28**, 205-12 (1958)
177. Brown, J. B., Bulbrook, R. D., and Greenwood, F. C., *J. Endocrinol.*, **16**, 41-48 (1957)
178. Brown, J. B., Bulbrook, R. D., and Greenwood, F. C., *J. Endocrinol.*, **16**, 49-56 (1957)
179. Muratorio, J. R., Tommasino, P. O., and Staffieri, J. J., *Proc. 4th Panam. Congr. Endocrinol.*, 299 (1957)
180. Furuhjelm, M., and Waller, R., *Acta Endocrinol.*, **27**, 482-92 (1958)
181. Bulbrook, R. D., Greenwood, F. C., and Williams, P. C., *J. Endocrinol.*, **15**, 206-10 (1957)
182. Slaunwhite, W. R., Jr., and Sandberg, A. A., *Endocrinology*, **62**, 283-86 (1958)
183. Dao, T. L., *Endocrinology*, **61**, 242-55 (1957)
184. Berli, R. R., Staffieri, J. J., and Tommasino, P. O., *Proc. 4th Panam. Congr. Endocrinol.*, 207 (1957)
185. Smith, O. W., and Blackham, N. N., *Acta Endocrinol.*, **25**, 133-60 (1957)
186. Junkmann, K., *Recent Progr. in Hormone Research*, **13**, 389-419 (1957)
187. Gurtman, A. I., Andrada, J. A., Blatt, M. H. G., Epstein, J. A., and Kupperman, H. S., *Obstet. and Gynecol.*, **10**, 261-65 (1957)
188. Gurtman, A. I., Andrada, J. A., Blatt, M. H. G., and Kupperman, H. S., *Proc. 4th Panam. Congr. Endocrinol.*, 261 (1957)
189. Trillinger, K. G., and Westman, A., *Acta Endocrinol.*, **25**, 113-19 (1957)
190. Furuya, H., Deguchi, K., Shima, M., *Am. J. Obstet. Gynecol.*, **74**, 635-50 (1957)
191. Kistner, R. W., and Duncan, C. J., *Obstet. and Gynecol.*, **10**, 68-72 (1957)
192. Gross, S. J., Lee, O. K., van Maeck, J., and Sims, E. A. H., *Obstet. and Gynecol.*, **10**, 504-17 (1957)
193. Epstein, J. A., Vosburgh, L., Vesell, M., and Kupperman, H. S., *Obstet. and Gynecol.*, **9**, 512-16 (1957)
194. Gross, S. J., Lee, O. K., Dery, L., and van Maeck, J., *Obstet. and Gynecol.*, **10**, 397-405 (1957)
195. Igarashi, M., *Fertility and Sterility*, **8**, 362-72 (1957)
196. Fels, E., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 80 (1957)
197. Brody, S., and Westman, A., *Acta Endocrinol.*, **27**, 493-98 (1958)
198. Telfer, M. A., and Hisaw, F. L., Jr., *Acta Endocrinol.*, **25**, 390-404 (1957)
199. Leatham, J. H., *Ann. N. Y. Acad. Sci.*, **75**, 463-71 (1958)
200. Elftman, H., *Endocrinology*, **62**, 410-15 (1958)
201. Cretius, K., *Gynaecologia*, **143**, 192-217 (1947)
202. Ville, C. A., *Ann. N. Y. Acad. Sci.*, **75**, 524-34 (1958)
203. Bever, A. T., *Ann. N. Y. Acad. Sci.*, **75**, 472-90 (1958)

204. Velardo, J. T., *Ann. N. Y. Acad. Sci.*, **75**, 441-60 (1958)
205. Wicks, A. E., and Segal, S. J., *Proc. Soc. Exptl. Biol. Med.*, **93**, 270-73 (1956)
206. Velardo, J. T., *Am. J. Physiol.*, **188**, 317-20 (1957)
207. Velardo, J. T., Hisaw, F. L., and Bever, A. T., *Endocrinology*, **59**, 165-69 (1956)
208. Velardo, J. T., *Am. J. Physiol.*, **186**, 468-70 (1956)
209. Luraschi, C., *Ann. di ostet. e ginecol.*, **78**, 1061-74 (1956)
210. Edgren, R. A., and Calhoun, D. W., *Am. J. Physiol.*, **189**, 355-57 (1957)
211. Overbeek, G. A., and de Visser, J., *Acta Endocrinol.*, **27**, 73-76 (1958)
212. Moore, N. W., and Robinson, T. J., *J. Endocrinol.*, **15**, 360-65 (1957)
213. Young, S., Bulbrook, R. D., and Greenwood, F. C., *Lancet*, **I**, 350-53 (1957)
214. Benson, G. K., Cowie, A. T., Cox, C. P., and Goldzweig, S. A., *J. Endocrinol.*, **15**, 126-44 (1957)
215. Höhn, E. O., *J. Endocrinol.*, **16**, 227-30 (1957)
216. Blair, S. M., Blair, P. B., and Daane, T. A., *Endocrinology*, **61**, 643-51 (1957)
217. Soliman, F. A., and Reineke, E. P., *Acta Endocrinol.*, **27**, 385-88 (1958)
218. Bogdanove, E. M., and Horn, E. H., *Endocrinology*, **62**, 97-99 (1958)
219. Suchowsky, G., *Acta Endocrinol.*, **27**, 225-37 (1958)
220. Clayton, B. E., and Hammant, J. E., *J. Endocrinol.*, **15**, 255-65 (1957)
221. Michael, R. P., and Scott, P. P., *J. Physiol. (London)*, **138**, 46P-47P (1957)
222. Howard, E., and Allen, J. C., *Endocrinology*, **60**, 785-96 (1957)
223. Berg, O. A., *Acta Endocrinol.*, **27**, 155-69 (1958)
224. Hartsook, E. W., and Magruder, N. D., *Am. J. Physiol.*, **190**, 255-58 (1957)
225. Glasser, S. R., *Am. J. Physiol.*, **189**, 441-46 (1957)
226. Berg, O. A., *Acta Endocrinol.*, **27**, 140-54 (1958)
227. Campbell, J. G., *J. Endocrinol.*, **15**, 339-45 (1957)
228. Campbell, J. G., *J. Endocrinol.*, **15**, 346-50 (1957)
229. Campbell, J. G., *J. Endocrinol.*, **15**, 351-54 (1957)
230. Johnson, E., *J. Endocrinol.*, **16**, 351-59 (1958)
231. Houssay, B. A., *Proc. Latin-Am. Assoc. Physiol. Sci.*, *1st Meeting*, 105 (1957)
232. Berezin, D., and von Studnitz, W., *Acta Endocrinol.*, **25**, 427-34 (1957)
233. Furman, R. H., and Howard, P. R., *Ann. Internal Med.*, **47**, 969-77 (1957)
234. Cembrano, J., Penna, A., Vial, M., and Mardones, J., *Proc. Latin-Am. Assoc. Physiol. Sci.*, *1st Meeting*, 52 (1957)
235. Malinow, M. R., Pellegrino, A. A., and Ramos, E. H., *Proc. Latin-Am. Assoc. Physiol. Sci.*, *1st Meeting*, 120 (1957)
236. Randall, C. L., Birtch, P. K., and Harkins, J. L., *Am. J. Obstet. Gynecol.*, **74**, 719-29 (1957)
237. Schneider, R. A., Costiloe, J. P., Howard, R. P., and Wolf, S., *J. Clin. Endocrinol. and Metabolism*, **18**, 379-90 (1958)
238. Albrieux, A. S., Saráchaga, D. R., and Rotundo, M. G., *Proc. 4th Panam. Congr. Endocrinol.*, 190 (1957)
239. von Studnitz, W., and Berezin, D., *Acta Endocrinol.*, **27**, 245-52 (1958)
240. Heller, J. H., Meier, R. M., Zucker, R., and Mast, G. W., *Endocrinology*, **61**, 235-41 (1957)
241. Ringer, R. K., Sturkie, P. D., and Weiss, H. S., *Am. J. Physiol.*, **190**, 54-56 (1957)
242. Greene, R. R., *Ann. N. Y. Acad. Sci.*, **75**, 586-99 (1958)
243. Meissner, W. A., Sommers, S. C., and Sherman, G., *Cancer*, **10**, 500-9 (1957)
244. Gardner, W. U., *Ann. N. Y. Acad. Sci.*, **75**, 543-64 (1958)
245. Lipschutz, A., *4th Panam. Congr. Endocrinol.*, main lecture, 76-77 (1957)

246. Forbes, T. R., and Hooker, C. W., *Endocrinology*, **61**, 281-86 (1957)
247. Forbes, T. R., *Endocrinology*, **61**, 593-94 (1957)
248. Edgar, D. G., and Ronaldson, J. W., *J. Endocrinol.*, **16**, 378, 384 (1958)
249. Short, R. V., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 362-75 (1957)
250. Short, R. V., *J. Endocrinol.*, **16**, 415-25 (1958)
251. Short, R. V., *J. Endocrinol.*, **16**, 426-28 (1958)
252. Ghilain, A., *Bull. fédération socs. gynécol. et obstét. langue franç.*, **9**, No. 1 bis, 51-54 (1957)
253. Davis, M. E., and Plotz, E. J., *Fertility and Sterility*, **8**, 603-18 (1957)
254. Plotz, J., *Geburtshilfe u. Frauenheilk.*, **17**, 595-610 (1957)
255. Davis, M. E., and Plotz, E. J., *Recent Progress in Hormone Research*, **13**, 347-79 (1957)
256. Zander, J., Forbes, T. R., von Münstermann, A. M., and Neher, R., *J. Clin. Endocrinol. and Metabolism*, **18**, 337-53 (1958)
257. Gorski, J., Domínguez, O. V., Samuels, L. T., and Erb, R. E., *Endocrinology*, **62**, 234-35 (1958)
258. Zander, J., *Klin. Wochschr.*, **35**, 1101 (1957)
259. Pearlman, W. H., *Biochem. J.*, **65**, 7P (1957)
260. Rappoport, W. J., Goldstein, B., and Haskins, A. L., *Obstet. and Gynecol.*, **9**, 497-504 (1957)
261. Sandberg, A. A., and Slaunwhite, W. R., Jr., *J. Clin. Endocrinol. and Metabolism*, **18**, 253-65 (1958)
262. Berliner, M. L., Berliner, D. L., and Dougherty, T. F., *J. Clin. Endocrinol. and Metabolism*, **18**, 109-14 (1958)
263. Figueroa, S., and Lipschutz, A., *Endocrinology*, **61**, 657-60 (1957)
264. Pumeau Delille, G., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 144 (1957)
265. Bergstrand, C. G., and Gemzell, C. A., *J. Clin. Endocrinol. and Metabolism*, **17**, 870-77 (1957)
266. Klopper, A. I., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 504-11 (1957)
267. Russell, C. S., Paine, C. G., Coyle, M. G., and Dewhurst, C. J., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 649-67 (1957)
268. Klopper, A., Strong, J. A., and Cook, L. R., *J. Endocrinol.*, **15**, 180-89 (1957)
269. Herrmann, W., and Silverman, L., *J. Clin. Endocrinol. and Metabolism*, **17**, 1482-85 (1957)
270. Langecker, H., *Acta Endocrinol.*, **28**, 148-52 (1958)
271. Wray, P. M., and Russell, C. S., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 526-30 (1957)
272. Eberlein, W. R., and Bongiovanni, A. M., *J. Clin. Endocrinol. and Metabolism*, **18**, 300-9 (1958)
273. Eichler, L. R., and Bradbury, J. T., *Endocrinology*, **61**, 204-5 (1957)
274. Stern, M. I., *J. Endocrinol.*, **16**, 180-88 (1957)
275. Willemse, C. H., and Paesi, F. J. A., *Acta Endocrinol.*, **27**, 59-63 (1958)
276. Pincus, G., Tamotsu, M., Merrill, A. P., and Longo, P., *Endocrinology*, **61**, 528-33 (1957)
277. Velardo, J. T., *Am. J. Physiol.*, **190**, 408-12 (1957)
278. Whitten, W. K., *J. Endocrinol.*, **16**, 80-85 (1957)
279. Vlyssidés, Z., and Kastrisios, E., *Gynécol. et obstét.*, **56**, 74-78 (1957)
280. Davis, E., and Wied, G. L., *Geburtshilfe u. Frauenheilk.*, **17**, 916-28 (1957)
281. Reifenstein, E. C., Jr., *Fertility and Sterility*, **8**, 50-79 (1957)

282. Finkler, R. S., *Fertility and Sterility*, **8**, 323-32 (1957)
283. Davis, M. E., and Wied, G. L., *J. Clin. Endocrinol. and Metabolism*, **17**, 1237-44 (1957)
284. Suchowsky, G., and Junkmann, K., *Acta Endocrinol.*, **28**, 129-31 (1958)
285. Lipschutz, A., Jadrijevic, D., Mardones, E., Figueroa, S., and Girardi, S., *J. Endocrinol.*, **15**, 248-54 (1957)
286. Fried, J., Borman, A., and Kessler, W. B., *Proc. 4th Panam. Congr. Endocrinol.*, 249-50 (1957)
287. Wied, G. L., and Davis, M. E., *Obstet. and Gynecol.*, **10**, 411-17 (1957)
288. Engel, C. R., and Noble, R. L., *Endocrinology*, **61**, 318-21 (1957)
289. Lipschutz, A., and Figueroa, S., *Acta Endocrinol.*, **26**, 371-76 (1957)
290. Zaffaroni, A., *Proc. 4th Panam. Congr. Endocrinol.*, 175 (1957)
291. Rock, J., García, C. R., and Pincus, G., *Recent Progr. in Hormone Research*, **13**, 323-39 (1957)
292. Kaiser, R., *Geburtshilfe u. Frauenheilk.*, **17**, 24-37 (1957)
293. Ferin, J., *J. Clin. Endocrinol. and Metabolism*, **17**, 1252-55 (1957)
294. Ferin, J., *Geburtshilfe u. Frauenheilk.*, **17**, 10-24 (1957)
295. Ferin, J., *Gynaecologia*, **144**, 37-42 (1957)
296. Pincus, G., *Proc. 4th Panam. Congr. Endocrinol.*, main lecture, 128-44 (1957)
297. Pincus, G., Rock, J., García, C. R., Rice-Wray, E., Paniagua, M., and Rodríguez, I., *Am. J. Obstet. Gynecol.*, **75**, 1333-46 (1958)
298. García, C. R., Pincus, G., and Rock, J., *Am. J. Obstet. Gynecol.*, **75**, 82-97 (1958)
299. Saunders, F. J., Edgren, R. A., and Drill, V. A., *Endocrinology*, **60**, 804-5 (1957)
300. Edgren, R. A., *Endocrinology*, **62**, 689-93 (1958)
301. Jadrijevic, D., Girardi, S., and Lipschutz, A., *Proc. 4th Panam. Congr. Endocrinol.*, 276 (1957)
302. Edgren, R. A., *Acta Endocrinol.*, **25**, 365-70 (1957)
303. Spencer, H., Berger, E., Charles, M. L., Gottesman, E. D., and Laszlo, D., *J. Clin. Endocrinol. and Metabolism*, **17**, 975-84 (1957)
304. McSwiney, R. R., and Prunty, F. T. G., *J. Endocrinol.*, **16**, 28-40 (1957)
305. Bur, G. E., and Montuori, E., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 36 (1957)
306. Goldman, J. N., Epstein, J. A., and Kupperman, H. S., *Endocrinology*, **61**, 166-72 (1957)
307. Kar, A. B., Karkun, J. N., and De, N. N., *Acta Endocrinol.*, **25**, 238-48 (1957)
308. Brooks, R. V., and Prunty, F. T. G., *J. Endocrinol.*, **15**, 385-92 (1957)
309. Ranney, R. E., and Drill, V. A., *Endocrinology*, **61**, 476-77 (1957)
310. Peters, J. H., Randall, A. H., Jr., Mendeloff, J., Peace, R., Coberly, J. C., and Hurley, M. B., *J. Clin. Endocrinol. and Metabolism*, **18**, 114-15 (1958)
311. Lutwak-Mann, C., and Adams, C. E., *Acta Endocrinol.*, **25**, 405-11 (1957)
312. Borth, R., and Stamm, O., *Bull. fédération socs. gynécol. et obstét. langue franç.*, **9**, No. 1 bis, 130-32 (1957)
313. Dorfman, R. I., *La fonction endocrine du testicule*, 134-38 (Masson et Cie., Paris, France, 532 pp., 1957)
314. Brinck-Johnsen, T., and Eik-Nes, K., *Endocrinology*, **61**, 676-83 (1957)
315. Migeon, C. J., Keller, A. R., Lawrence, B., and Shepard, T. H., *J. Clin. Endocrinol. and Metabolism*, **17**, 1051-62 (1957)
316. Poore, W., and Hollander, V. P., *Endocrinology*, **61**, 652-56 (1957)
317. Munson, P. L., and Sheps, M. C., *Endocrinology*, **62**, 173-88 (1958)

318. Schopman, W., Huis in 't Veld, L. G., Van Der Vies, J., and Lampe-Hintzen, D. A. V. M., *Acta Endocrinol.*, **28**, 153-62 (1958)
319. Morat6 Manaro, J., and Spremolla, F., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 129 (1957)
320. Edwards, R. W. H., and Kellie, A. E., *Acta Endocrinol.*, **27**, 262-74 (1958)
321. Tamm, J., Beckmann, I., and Voigt, K. D., *Acta Endocrinol.*, **27**, 292-302 (1958)
322. Hurlock, B., and Talalay, P., *Endocrinology*, **62**, 201-15 (1958)
323. Sobel, C., Golub, O. J., Henry, R. J., Jacobs, S. L., and Basu, G. K., *J. Clin. Endocrinol. and Metabolism*, **18**, 208-21 (1958)
324. Golub, O. J., Sobel, C., and Henry, R. J., *J. Clin. Endocrinol. and Metabolism*, **18**, 522-30 (1958)
325. Holtz, A. H., *Acta Endocrinol.*, **26**, 75-90 (1957)
326. Knisely, W. H., Grunt, J. A., and Berry, R. J., *Am. J. Physiol.*, **189**, 437-40 (1957)
327. Nimni, M. E., and Geiger, E., *Endocrinology*, **61**, 753-57 (1957)
328. von Studnitz, W., and Nyman, M., *J. Clin. Endocrinol. and Metabolism*, **17**, 910-12 (1957)
329. Berezin, D., and von Studnitz, W., *Acta Endocrinol.*, **25**, 435-44 (1957)
330. Kochakian, C. D., and Tillotson, C., *Am. J. Physiol.*, **189**, 425-27 (1957)
331. Keitel, H. G., and Sherer, M. G., *J. Clin. Endocrinol. and Metabolism*, **17**, 854-61 (1957)
332. Rindani, T. H., *Am. J. Physiol.*, **193**, 73-74 (1958)
333. Kalant, O. J., and Sellers, E. A., *Endocrinology*, **61**, 264-70 (1957)
334. Kalant, O. J., *Endocrinology*, **62**, 237-44 (1958)
335. Rinne, U. K., and Näättänen, E. K., *Acta Endocrinol.*, **27**, 423-31 (1958)
336. Rinne, U. K., and Näättänen, E. K., *Acta Endocrinol.*, **27**, 415-22 (1958)
337. Roy, S. N., Karkun, J. N., and Sur, R. N., *Acta Endocrinol.*, **27**, 216-24 (1958)
338. Szirmai, J. A., *Acta Endocrinol.*, **25**, 225-37 (1957)
339. Ebling, F. J., *J. Endocrinol.*, **15**, 297-306 (1957)
340. Sala, G., Baldratti, G., and Ronchi, R., *Proc. 4th Panam. Congr. Endocrinol.*, 333 (1957)
341. Montuori, E., Bur, G. E., Grinberg, R., and Oliveto, M., *Proc. 4th Panam. Congr. Endocrinol.*, 297 (1957)
342. Montuori, E., Bur, G. E., Grinberg, R., and Oliveto, M., *Proc. 4th Panam. Congr. Endocrinol.*, 299 (1957)
343. Montuori, E., Bur, G. E., Grinberg, R., and Oliveto, M., *Proc. 4th Panam. Congr. Endocrinol.*, 298 (1957)
344. Clayton, B. E., and Prunty, F. T. G., *J. Endocrinol.*, **17**, 29-34 (1958)
345. Randall, L. O., and Selitto, J. J., *Endocrinology*, **62**, 693-95 (1958)
346. Gillman, T., and Naidoo, S. S., *Endocrinology*, **62**, 92-97 (1958)
347. Stange, H. H., and Dittmann, M., *Zentr. Gynäkol.*, **79**, 729-45 (1957)
348. György, G., Laszlo, J., and Feher, L., *Zentr. Gynäkol.*, **79**, 1867-77 (1957)
349. Gitsch, E., *Endocrinology*, **62**, 391-99 (1958)
350. Caravaglios, R., and Cilotti, R., *J. Endocrinol.*, **15**, 273-78 (1957)
351. Zachariae, F., *Acta Endocrinol.*, **26**, 215-23 (1957)
352. Jensen, C. E., and Zachariae, F., *Acta Endocrinol.*, **27**, 356-68 (1958)
353. Zachariae, F., and Jensen, C. E., *Acta Endocrinol.*, **27**, 343-55 (1958)
354. Zachariae, F., *Acta Endocrinol.*, **27**, 339-42 (1958)

355. Parsons, L., MacMillan, H. J., and Whittaker, J. O., *Am. J. Obstet. Gynecol.*, **75**, 121-31 (1958)
356. Birnberg, C. H., Kurzrok, R., and Laufer, A., *J. Am. Med. Assoc.*, **166**, 1174-75 (1958)
357. de Allende, I. L. C., *Rev. arg. endocrinol. y metabolismo*, **3**, 365-75 (1957)
358. Arronet, G. H., *Fertility and Sterility*, **8**, 301-22 (1957)
359. Epstein, J. A., Kupperman, H. S., Blatt, M. H. G., and Stone, A., *Proc. 4th Panam. Congr. Endocrinol.*, 240-41 (1957)
360. Kupperman, H. S., Epstein, J. A., Blatt, M. H. G., and Stone, A., *Am. J. Obstet. Gynecol.*, **75**, 301-9 (1958)
361. van Wagenen, G., and Simpson, M. E., *Endocrinology*, **61**, 316-18 (1957)
362. Fowler, R. E., and Edwards, R. G., *J. Endocrinol.*, **15**, 374-84 (1957)
363. Cullen, M., *Proc. 4th Panam. Congr. Endocrinol.*, 225-26 (1957)
364. Chang, C., and Witschi, E., *Endocrinology*, **61**, 514-19 (1957)
365. Shettles, L. B., *Obstet. and Gynecol.*, **10**, 359-65 (1957)
366. Ingram, D. L., *J. Endocrinol.*, **17**, 81-90 (1958)
367. Rugh, R., and Wolff, J., *Fertility and Sterility*, **8**, 428-37 (1957)
368. Ingram, D. L., and Mandl, A. M., *J. Endocrinol.*, **17**, 1-12 (1958)
369. Johnson, D. C., *Endocrinology*, **62**, 340-47 (1958)
370. Green, S. H., *J. Endocrinol.*, **15**, 327-31 (1957)
371. Birnberg, C. H., and Abitbol, M. M., *Obstet. and Gynecol.*, **10**, 366-70 (1957)
372. Stone, M. L., Sedlis, A., and Zuckerman, M., *Am. J. Obstet. Gynecol.*, **76**, 544-49 (1958)
373. Eichner, E., Herman, I., Kritzer, L., Platock, G. M., and Rubinstein, L., *Ann. N. Y. Acad. Sci.*, **75**, 1016 (1958)
374. McGaughey, H. S., Jr., Corey, E. L., and Thornton, W. N., Jr., *Am. J. Obstet. Gynecol.*, **75**, 23-27 (1958)
375. Cullen, B. M., and Harkness, R. D., *J. Physiol. (London)*, **140**, 46P-47P (1958)
376. Rezek, G. H., *Ann. N. Y. Acad. Sci.*, **75**, 995-97 (1958)
377. Decker, W. H., Thwaite, W., Bordat, S., Kayser, R., Harami, T., and Campbell, J., *Obstet. and Gynecol.*, **12**, 37-46 (1958)
378. Kelly, J. V., and Posse, N., *Obstet. and Gynecol.*, **8**, 531-35 (1956)
379. Posse, N., and Kelly, J. V., *Surg., Gynecol. Obstet.*, **103**, 687-94 (1956)
380. Kupperman, H. S., Rosenberg, D., and Cutler, A., *Ann. N. Y. Acad. Sci.*, **75**, 1003-10 (1958)
381. Jablonski, W. J. A., and Velardo, J. T., *Endocrinology*, **61**, 474-75 (1957)
382. Zarrow, M. X., and Brennan, D. M., *Ann. N. Y. Acad. Sci.*, **75**, 981-90 (1958)
383. Steinetz, B. G., Beach, V. L., Blye, R. P., and Kroc, R. L., *Endocrinology*, **61**, 287-92 (1957)
384. Frieden, E. H., *Endocrinology*, **62**, 41-46 (1958)
385. Gerard, G., and Poletti, H., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, **89** (1957)
386. Nilsson, O., and Hellström, K. E., *Acta Obstet. Gynecol. Scand.*, **36**, 340-46 (1957)
387. Borell, U., Nilsson, O., and Westman, A., *Acta Obstet. Gynecol. Scand.*, **36**, 22-28 (1957)
388. Black, D. L., and Asdell, S. A., *Am. J. Physiol.*, **192**, 63-68 (1958)
389. Westman, A., *Acta Obstet. Gynecol. Scand.*, **36**, 1-21 (1957)
390. Hartman, C. G., and Stavorski, J., *Fertility and Sterility*, **8**, 555-57 (1957)
391. Stavorski, J., and Hartman, C. G., *Obstet. and Gynecol.*, **11**, 622-39 (1958)
392. Gruenwald, P., *Ann. N. Y. Acad. Sci.*, **75**, 436-40 (1958)

393. Crottogini, J. J., Pose, S. V., and Caldeyro-Barcia, R., *Excerpta Medica*, X, 22 (2nd World Congr. Intern. Fed. Gynecol. Obstet., free communication, 1958)
394. Johnson, T. H., *Am. J. Obstet. Gynecol.*, **75**, 240-48 (1958)
395. Rosa, C. G., and Velardo, J. T., *Nature*, **181**, 348-49 (1958)
396. Bartelmez, G. W., *Am. J. Obstet. Gynecol.*, **74**, 931-55 (1957)
397. Rumbolz, W. L., and Greene, E. G., *Am. J. Obstet. Gynecol.*, **73**, 992-97 (1957)
398. Foraker, A. G., *Am. J. Obstet. Gynecol.*, **74**, 405-12 (1957)
399. Homburger, F., Tregier, A., and Grossman, M. S., *Endocrinology*, **61**, 634-42 (1957)
400. Homburger, F., and Tregier, A., *Endocrinology*, **61**, 627-33 (1957)
401. Olds, D., and VanDemark, N. L., *Fertility and Sterility*, **8**, 345-54 (1957)
402. Siliotti, I., and Marchetto, G., *Attualità obstet. e ginecol.*, **3**, 11-21 (1957)
403. Brody, S., *Acta Endocrinol.*, **27**, 377-84 (1958)
404. Kostyo, J. L., *Endocrinology*, **60**, 33-37 (1957)
405. Cretius, K., *Z. Geburtshilfe u. Gynäkol.*, **149**, 131-56 (1957)
406. Cretius, K., *Z. Geburtshilfe u. Gynäkol.*, **149**, 113-22 (1957)
407. Tenney, B., Parker, F., Jr., and Robbins, S. L., *Am. J. Obstet. Gynecol.*, **75**, 656-59 (1958)
408. Sandberg, F., Ingelman-Sundberg, A., Lindgren, L., and Rydén, G., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 334-41 (1957)
409. Youssef, A. F., *Am. J. Obstet. Gynecol.*, **75**, 1305-19 (1958)
410. Youssef, A. F., *Am. J. Obstet. Gynecol.*, **75**, 1320-32 (1958)
411. Ingelman-Sundberg, A., Lindgren, L., Rydén, G., and Sandberg, F., *Acta Obstet. Gynecol. Scand.*, **36**, 263-67 (1957)
412. Talbert, L. M., McGaughey, H. S., Jr., Corey, E. L., and Thornton, W. N., Jr., *Am. J. Obstet. Gynecol.*, **75**, 16-22 (1958)
413. Corey, E. L., McGaughey, H. S., Jr., and Thornton, W. N., Jr., *Am. J. Obstet. Gynecol.*, **74**, 473-83 (1957)
414. McGaughey, H. S., Jr., Corey, E. L., and Thornton, W. N., Jr., *Am. J. Obstet. Gynecol.*, **74**, 53-56 (1957)
415. Jung, H., *Arch. ges. Physiol.*, **263**, 19-26 (1956)
416. Jung, H., *Arch. ges. Physiol.*, **263**, 27-38 (1956)
417. Fluhmann, C. F., *Surg., Gynecol. Obstet.*, **106**, 715-23 (1958)
418. von Kaulla, K. N., Aikawa, J. K., Harms, P. D., and Winkle, W. T., *Fertility and Sterility*, **8**, 444-54 (1957)
419. Büssing, H. J., *Zentr. Gynäkol.*, **79**, 456-67 (1957)
420. Andreoli, C., and Della Porta, M., *J. Clin. Endocrinol. and Metabolism*, **17**, 913-14 (1957)
421. Klein, L., and Carey, J., *Am. J. Obstet. Gynecol.*, **74**, 956-67 (1957)
422. Chesley, L. C., and Hellman, L. M., *Am. J. Obstet. Gynecol.*, **74**, 582-90 (1957)
423. Fillios, L. C., Kaplan, R., Martin, R. S., and Stare, F. J., *Am. J. Physiol.*, **193**, 47-51 (1958)
424. Pathak, C. L., and Kahali, B. S., *J. Clin. Endocrinol. and Metabolism*, **17**, 862-69 (1957)
425. Savi, C., and Cigada, G., *Minerva ginecol.*, **9**, 161 (1957)
426. Nallar, R., *Proc. 4th Panam. Congr. Endocrinol.*, 301-2 (1957)
427. Masters, W. H., *Am. J. Obstet. Gynecol.*, **74**, 733-42 (1957)
428. Parks, R. D., Scheerer, P. P., and Greene, R. R., *Surg., Gynecol. Obstet.*, **106**, 413-20 (1958)
429. Cameron, C. B., *J. Endocrinol.*, **15**, 199-205 (1957)

430. Brown, J. B., and Marrian, G. F., *J. Endocrinol.*, **15**, 307-9 (1957)
431. Shelesnyak, M. C., *Recent Progress in Hormone Research*, 269-317 (Academic Press, Inc., New York, N. Y., 646 pp., 1957)
432. Böving, B. G., *Ann. N. Y. Acad. Sci.*, **75**, 700-25 (1958)
433. Blandau, R. J., and Rumery, R. E., *Fertility and Sterility*, **8**, 570-85 (1957)
434. Averill, R. L. W., and Rowson, L. E. A., *J. Endocrinol.*, **16**, 326-36 (1958)
435. Adams, C. E., *J. Endocrinol.*, **16**, 283-93 (1958)
436. Pinto, R. M., and Nallar, R., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 139-40 (1957)
437. Jackson, D., and Robson, J. M., *J. Endocrinol.*, **15**, 355-59 (1957)
438. Segal, S. J., and Nelson, W. O., *Proc. Soc. Exptl. Biol. Med.*, **98**, 431-36 (1958)
439. Rosen, F., Millman, N., Pavelek, J., and Rosenberg, B., *Am. J. Obstet. Gynecol.*, **74**, 314-17 (1957)
440. Frederiksen, T., *Acta Obstet. Gynecol. Scand.*, **37**, 86-96 (1958)
441. Huggett, A. St. G., *Ann. N. Y. Acad. Sci.*, **75**, 873-88 (1958)
442. Drieux, H., *Gynéc. et obstét.*, **56**, 365-72 (1957)
443. Amoroso, E. C., *Ann. N. Y. Acad. Sci.*, **75**, 855-72 (1958)
444. Bardawil, W. A., Toy, B. L., and Hertig, A. T., *Am. J. Obstet. Gynecol.*, **75**, 708-17 (1958)
445. Patten, B. M., *Excerpta Medica, Sect. X*, 5 (2nd World Congr. Intern. Fed. Gynecol. Obstet., main lecture, 1958)
446. Page, E. W., *Am. J. Obstet. Gynecol.*, **74**, 705-15 (1957)
447. Wilkin, P., *Bull. federation socs. gynéc. et obstét. langue franç.*, **9**, No. 1 bis, 33-36 (1957)
448. Wilkin, P., and Bursztejn, M., *Bull. federation socs. gynéc. et obstét. langue franç.*, **9**, no. 1 bis, 37-41 (1957)
449. McGaughey, H. S., Jr., Jones, H. C., Jr., Talbert, L. M., and Parker Anslow, W., Jr., *Am. J. Obstet. Gynecol.*, **75**, 482-95 (1958)
450. Gruhn, I., *Zentr. Gynäkol.*, **79**, 1025-35 (1957)
451. Davies, J., *Am. J. Physiol.*, **188**, 21-24 (1957)
452. Knobil, E., *Ann. N. Y. Acad. Sci.*, **75**, 895-904 (1958)
453. Davies, J., and Lacy, P. L., *Am. J. Obstet. Gynecol.*, **74**, 514-17 (1957)
454. Migeon, C. J., Bertrand, J., Wall, P. E., Stempfel, R. S., and Prystowsky, H., *Ciba Foundation Colloq. on Endocrinol.*, **11**, 33-356 (1957)
455. Streaan, G. J., Gelfand, M. M., Pavilanis, V., and Sternberg, J., *Can. Med. Assoc. J.*, **77**, 315-23 (1957)
456. Potter, E. L., *Am. J. Obstet. Gynecol.*, **74**, 505-13 (1957)
457. Diczfalusy, E., Nilsson, L., and Westman, A., *Acta Endocrinol.*, **28**, 137-47 (1958)
458. Loring, J. M., and Vilee, C. A., *Acta Endocrinol.*, **25**, 371-76 (1957)
459. Diczfalusy, E., and Halla, M., *Acta Endocrinol.*, **27**, 303-13 (1958)
460. Lundin, P. M., and Holmdahl, S., *Acta Endocrinol.*, **26**, 388-94 (1957)
461. Little, B., and Rossi, E., *Endocrinology*, **61**, 109-11 (1957)
462. Zondek, B., and Goldberg, S., *J. Obstet. Gynecol. Brit. Empire*, **64**, 1-13 (1957)
463. Paine, C. G., *J. Obstet. Gynecol. Brit. Empire*, **64**, 668-72 (1957)
464. Danforth, D. N., and Hull, R. W., *Am. J. Obstet. Gynecol.*, **75**, 536-47 (1958)
465. Bautzmann, H., and Hertenstein, C., *Z. Zellforsch. u. mikroskop. Anat.*, **45**, 589-611 (1957)
466. Bautzmann, H., and Hertenstein, C., *Z. Zellforsch. u. mikroskop. Anat.*, **45**, 676-701 (1957)

467. Bautzmann, H., *Arch. Gynäkol.*, **187**, 519-45 (1956)
468. Davies, J., *Conf. on Gestation, Trans. 4th Conf.*, 11-70 (Josiah Macy Jr. Foundation, New York, N. Y., 216 pp., 1957)
469. Plentl, A., *Conf. on Gestation, Trans., 4th Conf.*, 71-114 (Josiah Macy Jr. Foundation, New York, N. Y., 216 pp., 1957)
470. Wirtschafter, Z. T., and Williams, D. W., *Am. J. Obstet. Gynecol.*, **74**, 309-13 (1957)
471. Wirtschafter, Z. T., and Williams, D. W., *Am. J. Obstet. Gynecol.*, **74**, 1022-28 (1957)
472. Wirtschafter, Z. T., *Am. J. Obstet. Gynecol.*, **75**, 718-23 (1957)
473. McKay, D. G., Richardson, M. V., and Hertig, A. T., *Am. J. Obstet. Gynecol.*, **75**, 699-707 (1958)
474. Alexander, D. P., Nixon, D. A., Widdas, W. F., and Wohlzogen, F. X., *J. Physiol. (London)*, **140**, 1-13 (1958)
475. Luse, S. A., *Conf. on Gestation, Trans. 4th Conf.*, 115-41 (Josiah Macy Jr. Foundation, New York, N. Y., 216 pp., 1957)
476. Villee, C. A., *Conf. on Physiol. of Prematurity, Trans. 2nd Conf.*, 9-76 (Josiah Macy Jr. Foundation, New York, N. Y., 160 pp., 1957)
477. Rogers Brambell, F. W., *Conf. on Gestation, Trans. 4th Conf.*, 143-201 (Josiah Macy Jr. Foundation, New York, N. Y., 216 pp., 1957)
478. Tyler, A., *The Beginnings of Embryonic Development*, 341-82 (American Association for the Advancement of Science, Washington, D. C., 1957)
479. Anderson, G. W., Commings, P., Alpert, S., Burke, F. G., Washington, J. A., and Maksim, G., *Med. Ann. Dist. Columbia*, **27**, 167-85 (1958)
480. Karlberg, P. J. E., *Conf. on Physiol. of Prematurity, Trans., 2nd Conf.*, 77-150 (Josiah Macy Jr. Foundation, New York, N. Y., 160 pp., 1957)
481. McKay, R. B., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 185-97 (1957)
482. Rooth, G., and Sjöstedt, S., *Acta Obstet. Gynecol. Scand.*, **36**, 374-81 (1957)
483. Bancroft-Livingston, G., and Neill, D. W., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 498-503 (1957)
484. Turnbull, E. P. N., and Baird, D., *Brit. Med. J.*, **II**, 1021-24 (1957)
485. Snyder, F. F., *Am. J. Obstet. Gynecol.*, **75**, 1231-43 (1958)
486. Snyder, F. F., *Obstet. and Gynecol.*, **11**, 599-616 (1958)
487. Paul, W. M., *Bull. Johns Hopkins Hosp.*, **99**, 357-61 (1956)
488. Kaplan, S., and Toyama, S., *Obstet. and Gynecol.*, **11**, 391-97 (1958)
489. Hon, E. H., and Hess, O. W., *Science*, **125**, 553-54 (1957)
490. Corner, G. W., Jr., and Stran, H. W., *Am. J. Obstet. Gynecol.*, **73**, 190-95 (1957)
491. Caldeyro-Barcia, R., *Proc. 3rd Latin-Am. Congr. Obstet. Gynecol.*, **2**, 31-38 (1958)
492. Hon, E. H., *Am. J. Obstet. Gynecol.*, **75**, 1215-30 (1958)
493. Westin, B., *Acta Paediat.*, **46**, 117-24 (1957)
494. Dawes, G. S., Handler, J. J., and Mott, J. C., *J. Physiol. (London)*, **138**, 9P-10P (1957)
495. Barboriak, J. J., De Bella, G., Setnikar, I., and Krehl, W. A., *Am. J. Physiol.*, **193**, 89-91 (1958)
496. Alexander, D. P., Nixon, D. A., Widdas, W. F., and Wohlzogen, F. X., *J. Physiol. (London)*, **140**, 14-22 (1958)
497. Hanon, F., Coquoin-Carnot, M., and Pignard, P., *Gynéc. et obstét.*, **56**, 64-73 (1957)
498. Lanman, J. T., *Endocrinology*, **61**, 684-91 (1957)
499. Nichols, J., Lescure, O. L., and Migeon, C. J., *J. Clin. Endocrinol. and Metabolism*, **18**, 444-52 (1958)

500. Kawahara, H., *J. Clin. Endocrinol. and Metabolism*, **18**, 325-27 (1958)
501. Murdoch, D., and Cope, I., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 382-84 (1957)
502. Cope, I., and Murdoch, D. J., *J. Obstet. Gynaecol. Brit. Empire*, **65**, 56-57 (1958)
503. Brodie, H. R., Cross, K. W., and Lomer, T. R., *J. Physiol. (London)*, **138**, 156-63 (1957)
504. Roberts, H., and Please, N. W., *J. Obstet. Gynaecol. Brit. Empire*, **65**, 33-40 (1958)
505. McKeown, T., and Record, R. G., *J. Endocrinol.*, **15**, 393-409 (1957)
506. McKeown, T., and Record, R. G., *J. Endocrinol.*, **15**, 423-29 (1957)
507. Dewar, A. D., *J. Endocrinol.*, **15**, 211-15 (1957)
508. Dewar, A. D., *J. Endocrinol.*, **15**, 216-29 (1957)
509. Dewar, A. D., *J. Endocrinol.*, **15**, 230-33 (1957)
510. Contopoulos, A. N., and Simpson, M. E., *Endocrinology*, **61**, 765-73 (1957)
511. Dieckmann, W. J., and Pottinger, R., *Am. J. Obstet. Gynecol.*, **74**, 816-30 (1957)
512. Paaby, P., *Acta Obstet. Gynecol. Scand.*, **37**, 69-85 (1958)
513. Newman, R. L., *Obstet. and Gynecol.*, **10**, 51-55 (1957)
514. De Alvarez, R. R., *Am. J. Obstet. Gynecol.*, **75**, 931-44 (1958)
515. Abrahams, V. C., and Pickford, M., *J. Physiol. (London)*, **141**, 527-34 (1958)
516. Burt, R. L., Donnelly, J. F., and Kimel, C. A., *Am. J. Obstet. Gynecol.*, **75**, 1197-1201 (1958)
517. MacGillivray, I., and Tovey, J. E., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 361-64 (1957)
518. Nelken, S., Bethoux, R., and Burthiault, R., *J. méd. Lyon*, **39**, 453-57 (1958)
519. Burt, R. L., and Kimel, C. A., *Am. J. Obstet. Gynecol.*, **74**, 295-99 (1957)
520. Burt, R. L., *Am. J. Obstet. Gynecol.*, **74**, 551-58 (1957)
521. Massad, F., Valiente, S., Ramirez, H., and Canesa, I., *Proc. 4th Panam. Congr. Endocrinol.*, **292** (1957)
522. Parry, H. B., and Shelley, H. J., *J. Physiol. (London)*, **140**, 48P-49P (1958)
523. Selye, H., *Am. J. Obstet. Gynecol.*, **74**, 289-94 (1957)
524. Little, B., Vance, V. K., and Rossi, E., *J. Clin. Endocrinol. and Metabolism*, **18**, 49-53 (1958)
525. Hunt, J. N., and Murray, F. A., *J. Obstet. Gynaecol. Brit. Empire*, **65**, 78-83 (1958)
526. Murray, F. A., Erskine, J. P., and Fielding, J., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 373-81 (1957)
527. Camilleri, A. P., *J. Obstet. Gynaecol. Brit. Empire*, **65**, 266-77 (1958)
528. Horger, L. M., and Zarrow, M. X., *Am. J. Physiol.*, **189**, 407-11 (1957)
529. Erdberg, M. R., Baker, H., Pasher, I., and Sobotka, H., *Am. J. Obstet. Gynecol.*, **75**, 767-72 (1958)
530. Dumont, M., and Karlin, R., *Gynéc. et obstét.*, **56**, 197-201 (1957)
531. Marcus, M. B., Cibley, L. J., Brandt, M. L., Millman, L., and Barlas, D., *Am. J. Obstet. Gynecol.*, **75**, 11-15 (1958)
532. Kennan, A. L., and Bell, W. N., *Am. J. Obstet. Gynecol.*, **73**, 57-64 (1957)
533. Stoffer, R. P., Koeke, I. A., Chesky, V. E., and Hellwig, C. A., *Am. J. Obstet. Gynecol.*, **74**, 300-8 (1957)
534. Werner, S. C., *Am. J. Obstet. Gynecol.*, **75**, 1193-96 (1958)
535. Feldman, J. D., *Am. J. Physiol.*, **192**, 273-78 (1958)
536. Hoar, R. M., and Young, W. C., *Am. J. Physiol.*, **190**, 425-28 (1957)

537. Lucas, J. J., Brunstad, G. E., and Fowler, S. H., *J. Endocrinol.*, **17**, 54-62 (1958)
538. Birke, G., Gemzell, C. A., Plantin, L. O., and Robbe, H., *Acta Endocrinol.*, **27**, 389-402 (1958)
539. Schüller, E., *Acta Endocrinol.*, **25**, 345-64 (1957)
540. Appleby, J. I., and Norymberski, J. K., *J. Endocrinol.*, **15**, 310-19 (1957)
541. Baulieu, E. E., de Vigan, M., Bricaire, H., and Jayle, M. F., *J. Clin. Endocrinol. and Metabolism*, **17**, 1478-81 (1957)
542. Laidlaw, J. C., Cohen, M., and Gornall, A. G., *J. Clin. Endocrinol. and Metabolism*, **18**, 222-25 (1958)
543. Schneider, G. T., Weed, J. C., and Bowers, C. Y., *Obstet. and Gynecol.*, **10**, 437-43 (1957)
544. Burgers, A. C. J., and Mighorst, J. C. A., *Acta Endocrinol.*, **25**, 249-58 (1957)
545. Bengtsson, L. P., *Am. J. Obstet. Gynecol.*, **74**, 484-93 (1957)
546. Bengtsson, L. P., *Am. J. Obstet. Gynecol.*, **74**, 518-20 (1957)
547. Hellman, L. M., Tricoli, V., and Gupta, O., *Am. J. Obst. Gynecol.*, **74**, 1018-21 (1957)
548. Turnbull, A. C., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 321-33 (1957)
549. Kaern, T., *Acta Obstet. Gynecol. Scand.*, **37**, 26-49 (1958)
550. Kelly, J. V., and Posse, N., *Am. J. Obstet. Gynecol.*, **74**, 124-33 (1957)
551. Smyth, C. N., *J. Physiol. (London)*, **137**, 3P-5P (1957)
552. Nixon, W. C. W., and Smyth, C. N., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 35-46 (1957)
553. Caldeyro-Barcia, R., Pose, S. V., and Alvarez, H., *Am. J. Obstet. Gynecol.*, **73**, 1238-54 (1957)
554. Pose, S. V., and Caldeyro-Barcia, R., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 142 (1957)
555. Cibils, L. A., *Proc. 2nd Uruguayan Congr. Obstet. and Gynec.*, **2**, 55-69 (1957)
556. Bainbridge, M. N., Nixon, W. C. W., and Smyth, C. N., *J. Obstet. Gynaecol. Brit. Empire*, **65**, 189-99 (1958)
557. Gemzell, C. A., Robbe, H., Stern, B., and Ström, G., *Acta Obstet. Gynecol. Scand.*, **36**, 75-92 (1957)
558. McCausland, A. M., and Holmes, F., *Western J. Surg., Obstet. and Gynecol.*, **65**, 220-31 (1957)
559. Stakemann, G., and Fuchs, F., *Excerpta Medica, Sec. X*, 43 (2nd World Congr. Intern. Fed. Gynecol. and Obstet., free communication, 1958)
560. Cort, R. L., and Cort, J. H., *Lancet*, **1**, 718-20 (1957)
561. Larks, S. D., and Dasgupta, K., *Am. J. Obstet. Gynecol.*, **75**, 1069-78 (1958)
562. Larks, S. D., *Nature*, **181**, 67-68 (1958)
563. Larks, S. D., Mackay, E. V., and Morton, D. G., *Am. J. Obstet. Gynecol.*, **75**, 1079-87 (1958)
564. Ciulla, U., *Ann. obstet. e ginecol.*, **79**, No. 7, 3-32 (1957)
565. Hon, E. H., and Davis, C. D., *Obstet. and Gynecol.*, **12**, 47-53 (1958)
566. Ciulla, U., *Ann. obstet. e ginecol.*, **79**, No. 8, 3-19 (1957)
567. Wright, H. P., Morris, N., Osborn, S. B., and Hart, A., *Am. J. Obstet. Gynecol.*, **75**, 3-10 (1958)
568. Johnson, T., and Clayton, C. G., *Brit. Med. J.*, **1**, 312-14 (1957)
569. Moore, P. T., and Myerscough, P. R., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 207-14 (1957)

570. Javert, C. T., *Spontaneous and Habitual Abortion* (McGraw-Hill Book Company, Inc., New York, N. Y., 450 pp., 1957)
571. Calderone, M. S., *Abortion in the United States* (Paul B. Hoeber, Inc., New York, N. Y., 224 pp., 1958)
572. Badawy, A. H., and Gabrawy, B., *J. Obst. Gynaecol. Brit. Empire*, **65**, 260-65 (1958)
573. Stephens, L. J., *Am. J. Obstet. Gynecol.*, **75**, 1255-61 (1958)
574. Goldzieher, J. W., and Benigno, B. B., *Am. J. Obstet. Gynecol.*, **75**, 1202-14 (1958)
575. *Bull. fédération socs. gynéc. et obstét. langue franc.*, 9/1 bis 5-162 (1957) (Proc. 17e. Congrès)
576. Cowie, A. T., *Progress in the Physiology of Farm Animals*, **III**, 907-61 (Butterworths Scientific Publications, London, Engl., pp. 743-1043, 1957)
577. Johansson, I., and Claesson, O., *Progress in the Physiology of Farm Animals*, **III**, 1005-43 (Butterworths Scientific Publications, London, Engl., pp. 743-1043, 1957)
578. Dodd, F. H., *Progress in the Physiology of Farm Animals*, **III**, 962-1004 (Butterworths Scientific Publications, London, Engl., pp. 743-1043, 1957)
579. Munford, R. E., *J. Endocrinol.*, **16**, 72-79 (1957)
580. Cowie, A. T., and Tindal, J. S., *J. Endocrinol.*, **16**, 403-14 (1958)
581. Poulton, B. R., and Reece, R. P., *Endocrinology*, **61**, 217-25 (1957)
582. Cowie, A. T., *J. Endocrinol.*, **16**, 135-47 (1957)
583. Donovan, B. T., and Van der Werff ten Bosch, J. J., *J. Physiol. (London)*, **137**, 410-20 (1957)
584. Hutton, J. B., *J. Endocrinol.*, **16**, 115-25 (1957)
585. Greenwald, G. S., *J. Endocrinol.*, **17**, 17-23 (1958)
586. Chitty, H., *J. Endocrinol.*, **15**, 279-83 (1957)
587. Naeslund, J., *Acta Obstet. Gynecol. Scand.*, **36**, 460-67 (1957)
588. Jost, A., *La fonction endocrine du testicule*, 9-18 (Masson et Cie., Paris, France, 532 pp., 1957)
589. Ånberg, Å., *Acta Obstet. Gynecol. Scand.*, **36**, Suppl. 2, 1-133 (1957)
590. Miller de Paiva, L., Vallejo-Freire, A., and Oliveira Filho, B., *Proc. 4th Panam. Congr. Endocrinol.*, 307-8 (1957)
591. Eugros, M. H., *Proc. 4th Panam. Congr. Endocrinol.*, 210-11 (1957)
592. Benoit, J., *La fonction endocrine du testicule*, 37-69 (Masson et Cie., Paris, France, 532 pp., 1957)
593. Verne, J., *La fonction endocrine du testicule*, 31-35 (Masson et Cie., Paris, France, 532 pp., 1957)
594. Savoie, J. C., *La fonction endocrine du testicule*, 19-30 (Masson et Cie., Paris, France, 532 pp., 1957)
595. Arrillaga, F., De la Balze, F. A., Vilar, O., Andrada, J. A., and Mancini, R. E., *Proc. 4th Panam. Congr. Endocrinol.*, 201-2 (1957)
596. Fels, E., and Bur, G., *Proc. 4th Panam. Congr. Endocrinol.*, 243-44 (1957)
597. Sundell, B., *Acta Endocrinol.*, **25**, 419-26 (1957)
598. Steinberger, E., and Nelson, W. O., *Endocrinology*, **60**, 105-17 (1957)
599. Forbes, A. P., *La fonction endocrine du testicule*, 109-20 (Masson et Cie., Paris, France, 532 pp., 1957)
600. Antliff, H. R., and Young, W. C., *Endocrinology*, **61**, 121-27 (1957)

601. Soulaireac, A., and Soulaireac, M. L., *La fonction spermatogénétique du testicule humain*, 73-89 (Masson et Cie., Paris, France, 415 pp., 1958)
602. Coujard, R., *La fonction spermatogénétique du testicule humain*, 91-105 (Masson et Cie., Paris, France, 415 pp., 1958)
603. Moricard, R., Gothie, S., and Guillon, G., *La fonction spermatogénétique du testicule humain*, 227-46 (Masson et Cie., Paris, France, 415 pp., 1958)
604. Burgos, M. H., and Ladman, A. J., *Endocrinology*, **61**, 20-34 (1957)
605. Burgos, M. H., and Ladman, A. J., *Proc. 4th Panam. Congr. Endocrinol.*, 211-12 (1957)
606. De Allende, I. L. C., and Astrada, J. J., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 26-27 (1957)
607. Shute, E. V., *J. Obstet. Gynaecol. Brit. Empire*, **64**, 390-95 (1957)
608. Roosen-Runge, E. C., Marberger, E., and Nelson, W. O., *Fertility and Sterility*, **8**, 203-19 (1957)
609. Mancini, R. E., Gerschenfeld, H. N., and Penhos, H., *Proc. 4th Panam. Congr. Endocrinol.*, 288 (1957)
610. Weir, D. R., and Leuchtenberger, C., *Fertility and Sterility*, **8**, 373-78 (1957)
611. Vaishwanar, P. S., *Am. J. Obstet. Gynecol.*, **75**, 139-43 (1958)
612. White, I. G., *Am. J. Physiol.*, **189**, 307-10 (1957)
613. Sherman, J. K., *Am. J. Physiol.*, **190**, 281-86 (1957)
614. Bernstein, G. S., *Fertility and Sterility*, **9**, 120-33 (1958)
615. Mann, T., *La fonction spermatogénétique du testicule humain*, 167-76 (Masson et Cie., Paris, France, 415 pp., 1958)
616. Bocci, A., and Barbanti, A., *Bull. fédération socs. gynéc. et obstét. langue franç.*, **9**, No. 1 bis, 591-94 (1957)
617. Cross, B. A., and Glover, T. D., *J. Endocrinol.*, **16**, 385-95 (1958)
618. Zeitlin, A. B., Cottrell, T. L., and Lloyd, F. A., *Fertility and Sterility*, **8**, 337-44 (1957)
619. Nelson, W. O., *Proc. 4th Panam. Congr. Endocrinol.*, main lecture, 99-111 (1957)
620. Witschi, E., Nelson, W. O., Segal, S. J., *J. Clin. Endocrinol. and Metabolism*, **17**, 737-53 (1957)
621. Nelson, W. O., *Fertility and Sterility*, **8**, 827-36 (1957)
622. Hamblen, E. C., *Am. J. Obstet. Gynecol.*, **74**, 1228-44 (1957)
623. Nelson, W. O., and Boccabella, R., *Fertility and Sterility*, **8**, 333-36 (1957)
624. Grumbach, M. M., Blanc, W. A., and Engle, E. T., *J. Clin. Endocrinol. and Metabolism*, **17**, 703-36 (1957)
625. Castro, N. M., Sasso, W. S., Trench, U. S., and Kerbaux, J., *Lancet*, **II**, 565 (1957)
626. Sohval, A. R., Gaines, J. A., and Strauss, L., *Ann. N. Y. Acad. Sci.*, **75**, 905-22 (1958)
627. Etcheverry, M., and Wais, S., *Proc. 4th Panam. Congr. Endocrinol.*, 242-43 (1957)
628. Etcheverry, M., and Wais, S., *Proc. 4th Panam. Congr. Endocrinol.*, 241-42 (1957)
629. Raboch, J., *J. Clin. Endocrinol. and Metabolism*, **17**, 1429-39 (1957)
630. Barr, M. L., *Excerpta Medica, Sect. X*, 3 (Proc. 2nd World Congr. Intern. Fed. Gyn. and Obst., Main lecture, 1958)
631. Greenblatt, R. B., *J. Clin. Endocrinol. and Metabolism*, **18**, 227-30 (1958)
632. Wilkins, L., *Proc. 4th Panam. Congr. Endocrinol.*, main lecture, 172-74 (1957)

- 633. Epps, D. R., Guerios, M. F. M., Coelho Neto, A. S., León, N., Assis, L. M., and Ulhoa Cintra, A. B. de, *Proc. 4th Panam. Congr. Endocrinol.*, 239-40 (1957)
- 634. Reforzo Membrives, J., Rocca, D. Z., Pinto, R. M., and Luchetti, S. E., *Proc. Latin-Am. Assoc. Physiol. Sci., 1st Meeting*, 150-51 (1957)
- 635. Di Paola, G., and Nogués, A. E., *Proc. 4th Panam. Congr. Endocrinol.*, 231 (1957)
- 636. Wilkins, L., Hones, H. H., Jr., Holman, G. H., and Stempfel, R. S., Jr., *J. Clin. Endocrinol. and Metabolism*, **18**, 559-85 (1958)
- 637. Pincus, G., and Hopkins, T. F., *Endocrinology*, **62**, 112-18 (1958)
- 638. Perrin, E. V., and Benirschke, K., *J. Clin. Endocrinol. and Metabolism*, **18**, 327-28 (1958)
- 639. Plate, W. P., *Acta Endocrinol.*, **26**, 101-3 (1957)
- 640. Roth, F., and Richter, R., *Zentr. Gynäkol.*, **79**, 1733-40 (1957)
- 641. Pedersen, K. O., *Phys. Colloid Chem.*, **51**, 164 (1947)

COMPARATIVE PHYSIOLOGY: ANNUAL REPRODUCTIVE CYCLES OF MARINE INVERTEBRATES^{1,2}

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Interest in the study of the physiology of reproduction is of relatively recent date and has developed largely in the last forty years. Interest in the physiology of reproduction of the invertebrates is of even more recent date. In fact, the most complete and up-to-date compendium of comparative animal physiology (1) omits the subject entirely. Yet it is known that in the invertebrate world exists a diversity of modes of reproduction from primitive to highly evolved, asexual³ as well as sexual (2, 3, 125, 126). Here is to be seen, so to speak, the evolution of the animal reproductive process, a procession of nature's fascinating experiments. Investigators have to some extent realized the opportunities for such studies, and many articles on the physiology of reproduction in invertebrates are widely scattered in the literature in a variety of journals. Neglect of discussion of the physiology of the reproductive processes of the invertebrates, then, has not been for lack of material but rather for lack of a synthesis of the existing literature.

It would be impossible in a single brief review to cover completely the physiology of invertebrate reproduction, desirable as it might be. Rather, this review considers only the annual reproductive cycles of marine invertebrates, with special emphasis on papers which include interesting viewpoints, experimental approaches, or provocative material.

A few general references which give considerable information on annual reproductive cycles of marine invertebrates are: the *Handbook of Biological Data* (4), in which is found a table containing breeding seasons and related data for invertebrates from various places; a book on methods for obtaining and handling marine eggs and embryos (5) of East Coast invertebrates (particularly for animals from the Woods Hole area); a paper (6) on New Zealand invertebrates; two books (7, 8) and several papers (9, 10) on West

¹ The information in this article covers the literature as far back as seemed feasible to go and includes citations up to August, 1958. Wherever possible, reference is given to key review articles which give references to older literature. If important or interesting work was omitted, it was unintentional.

² I am indebted to the staff of the Stanford Biological Libraries: Mr. F. Falconer, Chief Science Librarian, Miss Joanne Dandois, and Mr. Michael Sadoski without whose help it would have been impossible to make the extensive bibliographic search.

³ Asexual reproduction occurs in many groups of animals, among them the Protozoa, Porifera, Coelenterata, Platyhelminthes, Rotifera, Tunicata, and certain Annelida (Polychaeta). These are dealt with in general works (2, 3) on the invertebrates. Considerable research on polychaetes is referred to by Dürchon (123). *Dodecaceria*, one of the most interesting of the polychaetes, has a remarkable capacity for asexual reproduction, and Martin's paper (125) and references cited by him should be consulted for information.

Coast invertebrates; several papers on tropical invertebrates (11, 12, 51 to 57); and an extensive paper on Arctic invertebrates (13). The descriptive aspects of reproduction are well covered in such books as Borradaile & Potts (2) and, for many of the phyla, in Hyman's scholarly treatise on the invertebrates (3).

The topics of particular interest to be considered here are the methods most useful and advantageous for a study of reproductive cycles in marine invertebrates, the extent to which marine invertebrates show an annual reproductive period and the variation of this period with latitude, the factors which induce the reproductive cycle and the biochemical changes accompanying it, the specific conditions which induce spawning, and the nervous and hormonal mechanisms which are involved in reproduction.

METHODS FOR DETERMINING ANNUAL REPRODUCTIVE CYCLES

There have been many methods used in determining the course of the annual reproductive cycles for various species of marine invertebrates studied, and significant advances in such investigations would probably follow the development of satisfactory criteria for determining when an animal is in breeding condition and for characterizing the reproductive cycle. Spawning, numbers of larvae, the appearance of ripe gametes in gonads, the brooding of eggs, and the relative size of gonads have been used, at one time or another, by different investigators to define the reproductive season.

No one will gainsay the value of observations of spawning in the field as indicative of the culmination of the natural reproductive cycle. In fact, all such observations on spawning in nature should be published and made available to those interested in reproduction. Some workers (e.g., 13 to 21) have taken spawning as a criterion of ripeness, and by plotting the percentage of animals against time some of them have obtained a quantitative measure of reproductive capacity.

However, there are several objections to the use of spawning as a criterion of the breeding season. For some species, observations of spawning are difficult or impossible to make in the field or even in the laboratory. Also, observation of spawning may be quite misleading, especially when made in the laboratory, for reasons discussed below. Finally, the factors which induce spawning may be quite different from those which induce the annual reproductive cycle.

It is self-evident that the spawning of deep-sea animals cannot be observed in their natural haunts; this may become possible with the development of bathyscapes, but it is not likely to be practicable; the television camera offers more promise (161). Also, it is difficult to see natural spawning of intertidal species which spawn in the night, as do some chitons (14). In many species spawning has never been observed, presumably because no one was present at the right time. Furthermore, such observations as are made, for most species, are likely to be dependent upon chance, so that use of such data as the main or only criterion of the reproductive season is likely to limit the accumulation of information.

Observation of spawning in the laboratory [e.g. (20, 21)] is much more readily available and again gives interesting information for some species. If the conditions of the environment provided approximate the natural one and the animal has been living under laboratory conditions for some time before the spawn has occurred, the information is likely to be meaningful. However, unless the observations are checked against the spawning of the animals in nature, they could be misleading since in many species spawning could be prematurely induced by shock in even an immature animal (22), which ejects whatever loose cells are present in its gonads or ducts. On the other hand, shock sometimes prevents spawning even though the animal has ripe eggs or sperms (14, 15, 99). Furthermore, spawning in some species may be inhibited for long periods of time by laboratory conditions (23). Thus, spawning in the sea urchin, *Arbacia punctulata*, may be prevented by keeping it in running sea water in aquaria. It retains its gametes well into the winter, whereas it spawns in summer at Woods Hole, Massachusetts. The eggs finally cytolyze and are resorbed, without having been spawned in the aquaria.

Identification of breeding cycles with spawning is unfortunate since it confuses the two problems, especially in discussions as to what factors are causative. It is quite likely that the various factors such as shock, temperature, tides, moonlight, chemicals, etc., which trigger spawning by brief periods of action are quite different from those which induce the overall cycle of growth of the gonads and maturation of the gametes, which probably must act for more prolonged periods of time.

Another method which has been used in such studies on the reproductive cycles of marine invertebrates is daily gathering the larvae of a given species from a known volume of water. From the relative density of larval populations present at different times of the year, the breeding season in a given region can be ascertained provided the larvae are not selectively removed by enemies or by water currents. This method has been used extensively for studies on oysters in Europe (82) but has been severely criticized (86). In waters off the coast of Holland the larvae are shed about eight days after fertilization. Counts are made daily or as often as feasible and the information, under favorable conditions, gives a good idea of the relative amounts of spawn and development. This method has not been used as widely for other species of oyster (83) or for other species of invertebrates, since in many cases water currents move the larvae, making the counts erratic. However, these determinations would give interesting information concerning survival of the young and their distribution by water currents to investigators of such problems (153, 154, 169).

Another criterion for assessing the reproductive state of an animal is the appearance of the gametes when studied under the microscope [e.g. (24 to 29, 128, 147, 165)]. Many investigators have used fresh material for this purpose; others have fixed the gonads periodically and studied the mounted and stained sections of the material. In this way they have obtained detailed cytological information precisely defining the stages in the gonadal cycle.

Some have attempted to grade the progressive degree of ripeness of a testis or ovary in approximately the following way: (a) activity (growth or gametogenesis) beginning, (b) gametogenesis well along, (c) gonads containing ripe gametes, (d) free gametes present in gonads and ducts, (e) animal spawning (gonad partially spawned out), (f) gonads completely spawned out, and (g) gonads in intermediate or neutral state. The percentage of animals in each reproductive stage may then be plotted against time and a graph delineating the annual reproductive cycle is thus obtained. This method has much to recommend it and has given considerable information about the course of events in a number of species, especially the detailed cellular changes in the gonad which accompany the reproductive cycle.⁴

A variant of the above method has been used with a variety of crustacea. Because during the reproductive season the mature females carry the eggs (i.e., they are ovigerous) either in a brood pouch or attached to swimmerets, they can be easily detected (30 to 34). The percentage of such females "in berry" in a given population, determined periodically, may be plotted against the time to give a curve for the annual reproductive cycle (34). Usually the incubation time of the eggs is also determined and, in some studies, information is available as to how many times a female will re-berry herself during one reproductive season. This method has the advantage that the animal need not be killed for the observation; therefore the same animals may be used over and over again. However, it has the disadvantage that one does not know the cytological condition of the ovary or accessory organs during the reproductive season unless one sacrifices the animals as has been done in some cases (12).

Still another method used for assessing the reproductive condition of an animal is determination of the ratio of the gonad size to body weight (gonad index)⁵ (35, 36, 37). This method rests on the assumption that in individuals large enough to be mature a spent or immature gonad is small, whereas a ripe gonad is large and developed. A gonad which decreases suddenly is assumed to have just lost its gametes (spawned). Bits of gonadal tissue are examined to ascertain the degree of development of gametes and to see if active sperms are present in the male or mature eggs in the female. When possible, tests for the degree of fertilizability of the eggs are also made.

⁴ In hermaphroditic animals examination of the gonads and assessment of their state of development are perhaps most effectively carried out by this method, since it gives information about the sequence of events in the change from maleness to femaleness and the pattern of alternation between the two (58, 75, 80, 81, 85, 87, 100, 127, 160). The complexity introduced into annual reproductive cycles by hermaphroditism cannot be considered in this paper, interesting though it be.

⁵ The gonad index in some cases is the volume of the gonad determined by displacement of fluid in a graduated cylinder, divided by the wet weight of the animal, multiplied by 100 (35, 36), or in others, the weight of the gonad divided by the weight of the animal (166) multiplied by 100 (37) or the volume of the gonad divided by the volume of the animal (24).

This method makes possible plotting a gonad index, which is considered to be a measure of the average state of the reproductive population, against the time. The sweep of the reproductive cycle can thus be represented graphically. The method has the disadvantage that the specimens are sacrificed and continuous observations cannot, therefore, be made upon the same sample of individuals. Needless to say, it would be advantageous to couple this attack with observations of spawning (in nature and in the laboratory), as well as to make cytological studies of the tissues in order to follow the detailed changes in the gonads during the breeding cycle.

A variant of the above method has been applied to the abalones, because in this case the gonad is inseparable from the digestive gland. The distribution of tissues is traced and the area occupied by the reproductive tissue is determined for slices at various levels of the frozen visceral mass. From this an index of the degree of development of the gonads may be obtained. An increase in overall area occupied by gonads is considered to be a function of the increase in bulk of reproductive tissue (38). Still another possible variant of the gonad index method for the determination of the reproductive cycle is to find the relation between length of gonad and some linear dimension of the animal, that is, arm length of a starfish or radius of a sea urchin. This method does not seem to have had much application.

It must be indicated here that many of the observations available on the reproductive condition of marine invertebrates have been made at varying time intervals: daily, weekly, fortnightly, monthly, quarterly, or sporadically as the occasion allowed, and in the latter case especially during summer visits to marine laboratories. Assumptions must be made as to what happens during the time between observations. Probably only animals in the laboratory could be observed from day to day. In a few instances an investigator was able to use an isolated tide pool for daily observations for a limited period of time (14). When intertidal animals are being studied and they cannot endure laboratory conditions or small exposed tide pools, studies are likely to be limited to the time of low tide when the animals can be collected. Animals from deeper waters become unavailable in stormy weather, during which collections are impossible (59). Animals from the polar regions are often unobtainable during the cold parts of the year (13). In some cases, as in the study of oysters and barnacles, rafts are used containing the animals which can be kept in their natural environment and pulled up periodically for observation. This technique might profitably be used for the study of other invertebrates. In a sense, a submerged raft gives the advantages of the laboratory without removing the animals from its natural environment.

Most investigators agree that sampling should continue at least through a year rather than for a season judged, *a priori*, to be the likely reproductive season. For animals in less accessible habitats this has often been impossible. It is also generally agreed that observations are much more likely to be meaningful if made over a number of years. This is true because the onset and termination of the reproductive season of many marine invertebrates

have been found to vary considerably from year to year. Data, like those on oysters, gathered for twenty years at the Oosterschelde Laboratory in Holland have made possible many deductions not possible from data over a short span of time.

The size of a population tested for reproductive condition has also varied considerably. Some records in the literature tell of a few animals spawning, others of a single one. Some workers use a sample of ten animals, others twenty, and still others hundreds. Investigators agree that, if feasible, a large sample is preferable to a small one, considering the great individual variability in breeding condition often found for a sample of some marine invertebrates (36).

In order to have a more complete account of the annual reproductive cycle of a given species, some workers have studied it in relation to latitude and have demonstrated that the precise length, onset, and termination of the reproductive season usually appertains only to a given latitude and sometimes also to a given local set of conditions (23, 60, 62 to 66).

ANNUAL REPRODUCTIVE CYCLES

By "reproductive cycle" is meant one entire cycle, that is, the series of events from the time of activation, growth, and gametogenesis in the gonad to spawning of the gametes and recession of gonadal activity to a relatively sustained resting level, and including the duration of the rest period (that is, until the gonad is once again activated). In the case of continuously breeding animals such a cycle may occupy a period of a few weeks or several months and in the case of animals breeding twice a year, approximately six months, whereas in the case of an annual reproductive cycle it occupies an entire year. The entire reproductive cycle may be divided into a reproductive period (season), during which the gonad shows activity from its first initiation until spawning, and the vegetative period which follows reproduction and corresponds to the resting period of the gonad.

The reproductive period may be characterized by a series of gametogenic cycles each followed by spawning, and brief—rather than sustained—rest periods. In other words, more than one brood may be produced even if an annual reproductive cycle characterizes the species, provided that several gametogenic cycles occur during the reproductive period.

The spawning period is the time when the gametes are actually released. Spawning periods (seasons) therefore may occupy a small fraction of the time occupied by the entire reproductive period. Animals which release the spawn only by mating do so when the spawn is ready for transfer. The mating (breeding) period (season) in cohabiting animals therefore corresponds to the spawning periods of animals which release the gametes into the sea.

When studying the reproductive cycle in marine invertebrates one can build up an evolutionary transition from spawning of eggs and sperms into sea water (often synchronized) to pairing without copulation (40) to pseudocopulation (41) and to true copulation (2). Many coelenterates, echinoderms,

annelids, lamellibrachs, amphineurans, and some primitive chordates simply release their eggs into the sea; and such primitive larvae as the planula, pluteus, bipennaria, trochophore, veliger, and tornaria are the characteristic resulting planktotrophic larvae. Pseudocopulation is found in some worms which release their gametes into a common slime or pair to transfer sperm, even though the males lack an intromittent organ. Copulation in all higher invertebrates requires no comment. Pairing and copulating animals produce fewer eggs and often care for them or brood them.

A review of the literature leaves one with the feeling that marine invertebrates do not generally reproduce continuously throughout the year but rather do so in a restricted period. This is true for the polar, temperate, and even tropical regions although a distinctive pattern is found for each, the most limited breeding period being in the polar regions, the most extended in the tropics.

In Arctic and Antarctic polar species, spawning and breeding occurs for a limited period of time during the spring and summer when the plankton blooms occur and other conditions are somewhat more favorable for development and settling of the larvae or young. Sometimes populations are bimodal, suggesting two broods, but it is more likely that such cases will be resolved as was done for the arrow-worm, *Sagitta elegans arctica* (42, 43). The *Sagitta* young of the summer brood grow exceedingly slowly and are still immature at the beginning of the next summer, when they grow rapidly. During the second summer they become ready to breed. The population is thus always made up of two sizes of animals, one breeding in the summer, one not. Many Arctic invertebrates from the Point Barrow region probably also have but one brood a year (13) although some snails may have overlapping broods. On the other hand, the pelagic Arctic forms such as the ctenophores, arrow-worms, and jellyfish probably have several broods a year since larval forms, intermediate stages, and adults for all of these groups are quite often found in collections from one place at a given time (13).

Although spawning occurs for only a brief time in the polar regions, it is likely that the period of build-up of the gonads and formation of gametes covers a much wider span of the year. MacGinitie (13) points out that a given animal at Point Barrow probably begins accumulating oil reserves in summer a year before it will produce gametes. During the following winter and early spring, gametogenesis is probably proceeding, which makes possible the rapid maturation of the gametes when conditions favorable to spawning arise. Spawning and mating (breeding) are thus limited to a brief season in many polar forms but reproductive activities may cover a much larger fraction of the time of an annual reproductive cycle. Unfortunately, sampling in the polar regions has not yet been possible on an annual basis for a series of test species.

In temperate seas, spawning sometimes also occurs over a brief period of time, as in the case of some of the annelids known as palolo worms (99). In most invertebrates, however, spawning occurs over a much longer period

of time; and in a few cases it may last the greater part of the year, giving the impression that the animal is continuously breeding. Animals of brief, prolonged, or almost continuous spawning periods may occur side by side in the same environment (45, 150). Thus, in the Pacific Grove area of Monterey Bay the ochre starfish, *Pisaster ochraceus*, usually spawns in May; the purple sea urchin, *Strongylocentrotus purpuratus* spawns over several months and mature eggs and sperm may be obtained in a few individuals at least over a good part of the year, while the starfish, *Patiria minata*, has been said to spawn almost any time of the year although the maximal number of individuals spawn in spring and early summer (45).

Unquestionably, even those marine invertebrates which spawn during a limited period of time are in preparation for this event long in advance. In some of the annelids which spawn during very brief swarms, the posterior end of the worm usually develops reproductive organs, differentiates other characteristic structures and, as an epitoke, now separates from the anterior vegetative (atokous) portion, participates in the swarms that spawn, and dies after the gametes are released (99). A whole new sexual individual—the epitoke—is therefore induced preceding spawning in these species, although as a result of the drama of the swarm and spawn less consideration has been given to the environmental stimuli which might induce the development of the epitokes. The reproductive period is therefore not as brief as it appears to be.

In the ochre starfish, growth of the gonads is apparent long before spawning and the entire gonadal cycle from the beginning of growth to the emptying of the gonads may occupy many months (45). A low gonad index indicates that the animal has just spent or is in the postspawning quiescent stage, which lasts for several months. During the quiescent stage it is impossible by microscopic examination to distinguish between male and female, but other changes are visible, such as the growth of the pyloric or hepatic caecae. These fill up with food reserves and increase in size. Even before the gonad index has risen markedly, gametogenesis can be observed and sexes can be distinguished. As the gonad increases in size, the pyloric caecae shrink, the relation between the two being reciprocal. Gametogenesis and related changes have been described for many species of marine invertebrates (24 to 29, 128). Sometimes gametogenesis precedes spawning by many months as in the clam, *Cyprina islandica* (28) while in other cases it occurs about a month in advance as in the Pismo clam, *Tivela stultorum* (47).

An extended breeding season may mean that the individuals of a species are producing several successive broods during the year or that they are breeding asynchronously, i.e., some are in earlier stages of maturation, some are getting ready to spawn, some are spawning, and still others are already spent. If a large proportion of a population is successively spawning, several broods are being produced. However, even if only a small proportion of animals spawn at any one time, as appears to be the case in the purple sea urchin (36) during part of the year, several broods may still be produced by

a given individual but not synchronously with other individuals. By an ingenious technique Koehler (48) opened a window (which he could seal with wax) into the side of a sea urchin, *Strongylocentrotus lividus*. Through the window he could clip off a piece of sea urchin gonad from which he could determine the stage of development of the eggs. He found that at Naples, after eggs are shed, it takes six to eight weeks for a new set to develop. Fox (50) found new eggs in nine days at Roscoff. There is, therefore, no doubt that several broods of eggs could be produced by these urchins. Fox (49) working with the sea urchin, *Centrochinus (Diadema) setosa* of the Red Sea, showed that during one week a population was completely devoid of eggs after a general, well-synchronized spawn, and within a week of a new batch of eggs was detectable. Spawning occurs monthly in this species; therefore, full development of one "brood" presumably takes several weeks. An even shorter period of time has been claimed for re-formation of eggs in the purple urchin, *S. purpuratus*, according to Loeb (39), judging from successive and frequent spawns in most of the population under observation. Verification using Koehler's method would be most interesting since in the latter case the sea urchin lives at temperatures of about 12 to 15°C.

It is interesting to note that brooding of the young is a common habit of Arctic and Antarctic animals, pelagic existence of larvae being uncommon. Planktotrophic larvae would have to complete their development in five or six weeks of good weather at a temperature of 2 to 4°C., surprisingly enough, however, the few that have planktotrophic larvae are successful inasmuch as, in terms of numbers of individuals, they form a fair part of the population (44). Antarctic forms have presumably developed even further than Arctic forms in laying large eggs, in brooding, and in vivipary (44).

Species of marine invertebrates, from temperate seas, which have pelagic planktotrophic larvae presumably have short breeding seasons since the larvae depend upon the presence of appropriate plankton for survival (44). Spawning of such animals is likely to be synchronized at a favorable time in some manner rather than occurring sporadically over a longer period of time. Even where sporadic spawning occurs, the great mass of the population spawns within a fairly brief span of time (36). The value to the species of the continued spawning over many months, in addition to that of the major spawn, is an interesting evolutionary problem.

Species of marine invertebrates, in temperate seas, which produce yolky eggs or which brood their eggs are more likely to show a prolonged breeding season since the larvae are not likely to be dependent for as long a time upon the plankton, which is not always abundant here, as are the smaller pelagic planktotrophic larvae. Thus, females of various species of crustacea hold their eggs in brood pouches or attached to swimmerets. The eggs are yolky, relatively few in number, and the young are released only when they have developed into fair-sized larvae. As expected, many crustacea from temperate seas produce several broods during a prolonged breeding season. Brittle stars also produce a small number of eggs which they carry in a brood

chamber until the small starfishes are ready to fend for themselves. Many other groups of animals living in regions where pelagic larvae are likely to be carried off to sea also have a small number of eggs which they brood.

In the tropics, where planktonic food is presumably always available along the shores, one might expect the breeding season of invertebrates to be continual. While some reef-building corals breed periodically every month (11), this is not true of all corals or of other invertebrates, that is, in waters near Japan (51) or in the Caribbean (56). Off the Madras coast of India polychaetes occur throughout the year with a maximum in November and March when the richest phytoplankton occurs, and oysters breed all year although maximal breeding occurs during certain months as it does in many other invertebrates (52, 53). Brackish water animals in Madras reproduce throughout the year, some species every month, others during two or more reproductive seasons mainly related to rainfall (52, 53, 54). From the Philippines (55) come data indicating that in the warm seas the tendency is towards continuous breeding but with more intense activity during some seasons. While some forms on the Great Barrier Reef of Australia appear to breed continuously throughout the year, reproduction is periodic in many marine invertebrates, and a census of all the marine invertebrates studied shows that a majority of them spawn either exclusively or most intensely in the warmer months (11, 12, 57).

Invertebrates of tropical seas develop a great preponderance of pelagic planktotrophic larvae, 80 to 85 per cent of the species of marine invertebrates having larvae of this type as compared to 55 to 65 per cent in temperate seas and only 5 per cent in the Arctic (44). Considering that the ocean is full of hungry planktotrophic animals, the preponderance of pelagic plankton-eating larvae in the development of sea life attests to the effectiveness of these larvae in distributing a species.

Little is known of the life cycles of deep sea invertebrates (44). In the fragile sea urchin, *Allocentrotus fragilis*, which occurs at depths of approximately 90 to 840 m., a distinct breeding cycle has been demonstrated, the gonad increasing in size until about September or October and spawning occurring between January and April (59). Although temperature varies only 2°C. at the depths from which the samples were taken, food apparently varies in amount since urchins in most collections have little food in the gut, but when taken after a seasonal diatom bloom they are literally stuffed with diatoms and other material. The fragile urchin, being found only at moderate depths of the sea, still has a typical planktotrophic pelagic larva (59), as do other animals from such depths (153, 154). Invertebrates from great depths of the sea presumably do not have such larvae; what little is known about such forms indicates that they produce large eggs and that many brood their young (44).

From all the above observations it appears that the polar, temperate, and tropical seas each have a distinctive pattern of breeding season and type of larvae but some species of marine invertebrates in each category deviate from the pattern typical to their region.

One of the interesting facts which appear when the breeding season of a temperate species is studied at various latitudes is that the season becomes extended as one goes south. An example is *Arbacia punctulata*, the illustrious sea urchin of the Woods Hole area, where it has a breeding season which extends from the middle of June to the middle of August (23), while during May the gonads are small and it is often difficult to tell testes from ovaries and in late August and early September they are spawned out. On the other hand, *Arbacia* found further south at Beaufort, North Carolina, not only show an earlier season but also a longer one. Thus, the eggs may be ripe as early as January and good eggs are available well into May. Further south still in Alligator Harbor, Florida, *Arbacia* eggs are available from September to June and they are in prime condition in February, March, and April (23).

An even more striking case of change in breeding season with shift in latitude is that of the sea urchin, *Paracentrotus lividus*. At Roscoff, Brittany, the breeding season is from June to August. At Naples in the Mediterranean, however, it breeds throughout the year even though the water temperature is 26°C. in summer and 13°C. in winter (60). In the latter case, it would be especially interesting to make detailed quantitative and qualitative measurements of the reproductive cycle at various times during the year to find whether a period of maximal intensity of breeding effort might still be present.

Many other examples of this phenomenon may be found in the literature although only a few more examples are cited here in order to indicate that the phenomenon is not limited to echinoderms. The American oyster, *Crassostrea virginica*, spawns as early as April in the Gulf of Mexico (61, 152) while it spawns only in July and August in Nova Scotia (62). Somewhat similar results have been obtained for the European oyster (63), for the scallop, *Pecten maximus*, in British waters (64), for the razor clam, *Siliqua patula*, in the Pacific Northwest and Alaskan waters (65), for the copepod crustacean, *Calanus finmarchicus*, in British and Greenland waters (66), and for *Sagitta elegans* at different latitudes (43). In *Calanus* two or three broods may occur in warmer waters whereas only one occurs in those of the cold waters (66), and in *Sagitta* four to five broods in warmer waters, one in the Arctic (43).

FACTORS WHICH INDUCE THE BREEDING CONDITION

Endogenous versus exogenous factors.—It could be argued that the course of events which occur in a reproductive cycle is explainable in three ways: it could be the result of a series of endogenous or internal events which build up inside the organism, or it could be the result of the operation of various exogenous factors of the environment such as temperature, light, salinity, foods available, etc. acting upon a rather plastic organism, or it could be a combination of both.

The possibility that breeding of marine invertebrates might be regulated by a natural cycle of growth and development—a sort of biological clock—

seems to have received little attention and no experimental data are available either to prove or to disprove it. The length of the breeding cycle and its placement, however, could be looked upon as a function of the rate of growth, development, and maturation of the tissues of the gonads, ending in spawning and followed by a quiescent period; when the gonad once again begins to gather reserves, gametogenesis is again initiated in preparation for the next reproductive season.

If the reproductive cycle is regulated only endogenously, i.e., it is determined by how long it takes to accomplish each of the successive biological acts in the cycle, the cycle should operate with the same timing no matter where the animal is placed geographically. The very fact that the breeding season of a temperate form is prolonged with decreased latitude suggests that exogenous conditions have much to do with the timing of the events of the breeding cycle. Secondly, the fact that more broods than one may be found in warmer climates for species of animals which produce only one brood in the north (66), again underscores the importance of exogenous factors in the reproductive cycle.

Granted that exogenous factors do influence reproduction, one can test their relative importance very prettily by manipulating the environment to see whether it is possible to initiate more than one breeding season, in a single calendar year, in a species which normally shows one. Loosanoff & Davis (67) showed that by manipulating the temperature they could do just this with the American oyster, *C. virginica*. This species normally breeds once a year from June to mid-September near Long Island. By growing the oysters on a float they could move them from the harbor to the laboratory without violence to the animals other than the temperature change. Normally the oysters hibernate from the end of the breeding season until the beginning of the next spring. However, when the temperature was raised from 4°C. in the harbor to 20°C. in the laboratory, the oysters were induced to spawn in January or February and they discharged normal gametes which fertilized and developed into larvae. The temperature was again lowered to 4°C. by returning the float to Milford Harbor. They again hibernated, but between May and the middle of June they once more completed gametogenesis (16°C.) Similar though less extensive results were also obtained with the quahog, *Venus mercenaria* (67).

The fact that in polar regions, where for only a brief period of time conditions are favorable for growth and reproduction, breeding is indeed most strikingly seasonal, whereas in the tropics, where the environment is most constant, reproduction in many species tends to be almost continuous, argues for the importance of the exogenous factors in regulation of the reproductive cycle. On the other hand, presence of cyclic reproduction in the depths of the sea where conditions, other than possibly the availability of food, are probably relatively constant, emphasizes that endogenous components are very important. The endogenous drive for a reproductive cycle appears to be plastic to the extent that the precise pattern it takes depends

upon the external factors which entrain or time the endogenous events in such a way as to be effective for the survival of the species. Studies on reproductive cycles of animals kept under constant conditions of temperature, light, salinity, pH, food, etc. are much needed and would furnish crucial evidence.

Most of the experimental work done on the possible role of exogenous factors on the entrainment of the reproductive cycle is centered on temperature. The other factors such as light, salinity, food, etc. appear to have held little interest for research, accounting for the very scant literature. Yet it is possible that factors other than temperature may be of equal if not greater importance, at least in some instances. Let us look into the matter a little further before discussing temperature.

Light.—It is a well-known fact that in the plant world, photoperiodicity, or regulation of the time of induction of reproductive processes (that is, blooming) by changing daylength is of prime importance; a long-day plant blooms when the days become long in spring, while a short-day plant blooms when the days become short in autumn. Blooming can be induced out of its normal season in nature when the light period is artificially controlled (70). A similar phenomenon is found for many vertebrates (71) and terrestrial invertebrates (72). For example, the growth of the testes of some male birds is normally induced by the lengthening days of spring; birds subjected to such a photoperiod come into breeding condition even in dead winter when the temperature is well below freezing. However, evidence of photoperiodic control of reproduction in most marine invertebrates is still lacking and experimentation is scarce (77) although photic control of spawning has been studied (see section on this topic). A spring breeding season is well correlated, however, with increasing daylength whereas in autumn and winter breeders it is correlated with shortening days (45), and the longer breeding season of marine invertebrates at lower latitudes might well be photoperiodically controlled—but correlations alone are dangerous (136)—and the appropriate experiments have not yet been made [see, however (70, 137)]. The marine invertebrates present more difficulty for such experimentation since most of them do not tolerate laboratory living and must be studied in the field. However, at the present time several marine forms have been successfully kept in apparently healthy condition for prolonged periods of time in the laboratory or on floats which could be illuminated or darkened: oysters (73, 74), barnacles (76), an annelid worm (77, 124), some starfishes (78), and a sea urchin (79). Oysters, particularly, should make excellent material for studies of the influence of daylength upon breeding, since so much is known about their cytology (75, 80, 81, 82) and general biology (82, 83).

Food.—It is difficult to assess the importance of food to breeding cycles in invertebrates, and perhaps some confusion exists because spawning and the overall reproductive period are sometimes confused in discussions. It has been noted that the larvae of the ripe barnacle, *B. balanoides*, are lib-

erated in nature during the spring diatom outburst and that in the laboratory they are liberated even when the diatoms are supplied unseasonably early (in winter). Some chemical substance diffusing from the diatoms is suspected as the causative agent although it has not been identified (118). It seems unlikely that an immature or a spent organism without food reserves is likely to spawn when starved. An immature form needs the food to grow and to develop its gonads, and a mature but spent organism needs to build up its reserves before it can spawn again. On the other hand, since the reserves may be deposited a long time before the actual breeding season sets in, the relation between food and breeding is likely to be obscured and one may find in some cases that it might not be necessary for the animal to feed while becoming sexually mature, provided the maturing process is brief (68). However, if an animal is starved for a very long time before the breeding season it may not have the reserves to develop its gonads (69). In the starfish, *P. ochraceus*, the hepatic caecae store food during the rest period and they are depleted when the gonad grows, the size relationship between the two being reciprocal (45). If no reserves are put by, the gonad cannot mature. Similarly, the sea urchin, *S. purpuratus*, fails to breed when starved for a sufficiently long time before the breeding season, the gonad tissue being used to maintain the animal (45). The importance of food for larvae must also be taken into account as a factor of some importance and one which might account for timing of some reproductive periods although the causative factors and relationships are obscure (34, 59, 151). Studies on the relation between food and spawning and the breeding season indicate an interesting field for further investigations.

Salinity.—Spring rains diluting natural and artificial brine pools initiate reproductive cycles in many brine pool organisms (167, 168) although no study specifically directed to this problem seems to have appeared. Paul (52) has called attention to the close correlation between reproduction of some marine animals and the dilution of the water by winter rains in Madras Harbor, India. Pannikar & Aiyar (54) indicate the importance of dilution of brackish waters along the Indian coast to the reproduction of the animals. However, no reference to experimental determination of the effect of salinity on the reproductive cycle of marine invertebrates was found in the literature.

Temperature.—Marine organisms, living in bodies of water with high heat capacity because of their size, are essentially buffered against extremes of temperature. True, along the shores of the sea, temperatures may vary much more than in deeper waters, but never as much as on land. Furthermore, the thermal variations which occur in a body of water as large as the ocean are periodic and, while they differ from year to year, they are not the fitful changes characteristic of the air or of small bodies of water like a pond or a river. In the evolutionary sense, entrainment of the events of the reproductive cycle to seasonal temperature changes might therefore be a suitable device for marine invertebrates, even though unsuitable for fresh water or terrestrial organisms. At the same time, it would result in more

variability in breeding seasons such as is actually found in many cases than would entrainment with daylength.⁶

Orton (84) proposed that temperature is the most important factor for regulation of breeding in marine invertebrates. He has gone so far as to suggest that a definite temperature for breeding is a physiological constant for each marine species. To this is added the corollary that the breeding season is of longer duration and starts earlier in the year in the warmer parts of the range of the species. He points out that the European oyster, *Ostrea edulis*, begins to breed when the water temperature reaches 15 to 16°C. and continues to do so as long as the temperature remains above this value. At Naples it begins breeding several months before it does so in England; therefore, the breeding season is much longer in Naples than in England. Orton's suggestions are to some extent supported by Runnström (60) who holds that widespread species which breed in Arctic waters in midsummer do breed much earlier in the southern part of their range but at the same approximate temperature as in the north. Conversely, species which breed in the Mediterranean area in spring do so only in late summer farther north.

Considerable difference in opinion exists as to how temperature changes initiate reproduction. Some authors think that temperature acts as a trigger, attainment of a given temperature setting off reproduction. Others assume a time-temperature effect, in which a given temperature allows the gonads to ripen. Thus, if the water temperature rises slowly, the gonads may develop gametes at a lower temperature than when it rises rapidly.

Korringa (63) points out that Orton's view may well hold in a general way but as expressed above it is too sweeping. At the Oosterschelde Dutch center for oyster studies, where data for twenty years of oyster breeding are now available it is clear that oysters breed only if the water temperature is 15 or 16°C., but only after they have been maintained at this temperature for several weeks during which the gonads ripen. Furthermore, they stop breeding long before the water temperature has fallen below 15°C.; for example, breeding activity reaches its height between the last ten days of June and the first ten days of August after which it declines rapidly, ceasing in September even if the temperature at this time is over 18°C. and does not fall below the supposed species breeding limit of 15°C. until October 1. He further argues that, although temperature has some importance in regulating the annual reproductive cycle in that breeding can be induced out of season by appropriate temperatures (67), factors other than temperature are involved in the reproductive activity of oysters, as shown by the fact that once breeding has begun it is not governed or even influenced by temperature fluctuations alone. A rise in temperature caused by fair weather does

⁶ Upon a priori grounds one might expect that, since temperature on land and in fresh waters varies so extensively and fitfully, not only during the year but also from year to year, freshwater and terrestrial plants and animals would require control of reproduction by some means other than temperature. Some invariant factor for control of the reproductive cycle, almost a necessity in an evolutionary sense, is provided by daylength which for a given day of the year is the same, year after year.

not mean a large production of larvae. The "ups and downs" of spawning of oysters must therefore be regulated by something other than temperature (63).

Korringa's strongest criticism of the wider application of Orton's view is that perhaps different genotypes (races or varieties) of a species may exist in different localities and that spawning may be genetically controlled (63). Thus, northern races may grow and develop at a temperature and under other conditions which would prevent southern races from breeding. Furthermore, local conditions may have selected a race adapted to breeding under peculiar conditions. An example is a race of the oyster, *O. edulis*, in a locality of Norway where breeding occurs only when the temperature is 25°C. or over, whereas neighboring oysters outside this local bed breed when the temperature is 15°C. On the other hand, a race of the same oyster in the Bay of Biscay, off the southern coast of Spain, breeds in March or April when the temperature is only 13 to 14°C. whereas other races nearby require a higher temperature (63).

The evidence for different races in a species, each with its own temperature optimum for breeding, is indicated by work on the American oyster, *C. virginica* (63). It was thought that 20°C. was necessary for spawning but the Florida race in Apalachicola Bay requires 25°C. Loosanoff & Nomejko (138, 162) found that oysters (*C. virginica*) from different regions along the Atlantic coast, kept together in Long Island waters, showed similar gonadal development, but spawning of those from the north occurred earlier. This suggests that temperature determines growth of the gonads but that other factors determine the release of the gametes in this species, as if the mechanism by which these oysters adapt to their specific environment lay in genetic control of spawning reactions. On the other hand, Butler (157) claims that after a period of acclimatization northern oysters were synchronous with southern ones.

Crisp (68) has called attention to another type of temperature effect—induction of breeding by a low temperature. This had been noted by others and some pertinent references are given by Young (88, 89), but Crisp (68) tested the effect of temperature on the breeding of the cold-water barnacles, *Balanus balanoides* and *Balanus balanus*, which normally breed each year between November and February. When vegetative animals were kept in tanks at between 15 and 18°C. no copulatory activity or fertilization was observed. When kept between 3 and 10°C. for several months, breeding was induced. If kept at 15 to 18°C. after the eggs had developed, normal copulation, fertilization, and development occurred; therefore the high temperature did not inhibit these processes. Both species of barnacles require a period of cold for induction of breeding condition. They may even be starved without altering the course of events, provided eggs have already started developing in the ovary.

CYCLIC BIOCHEMICAL CHANGES ACCOMPANYING THE REPRODUCTIVE CYCLE

It is clearly indicated that the gonad is the locus of intensive biochemical synthesis at the time gametes are being formed (94). In the male large

amounts of nucleic acids are needed for the sperm heads, and in the female much lipid and protein is mobilized to be stored in the eggs. When reserves are stored in other organs preceding gametogenesis, transfer of these reserves to gonadal synthetic centers occurs at gametogenesis. Food reserves and their handling during breeding have been studied rather sparingly for so important a problem.

Some work has been done to determine the variation in chemical composition of decapod (164), mussel (90, 163), and oyster tissues (92, 145) in correlation with the reproductive cycle. The work on oysters has been critically reviewed by Korringa (82). Most interest has centered on the glycogen content of oysters (91) because a "fat" oyster ready for market is one rich in glycogen. Several investigators (92, 145) working with *Crassostrea gigas*, the Japanese oyster, found a considerable drop in glycogen, protein, fat, and ash content per oyster in summer during the breeding season. This is probably correlated with decline in total amount of flesh, since on the basis of unit weight of flesh the glycogen content did not decline although the spent gonads had considerably less glycogen and the mantle edges more (92). Humphrey (93), working with the Australian oyster, found considerable variation in glycogen content from oyster to oyster, but for a population the curve was smooth throughout the year. However, spawners showed a fall in glycogen content.

In the sea urchin, *S. purpuratus*, during the growth of the gonad from its inactive to the gravid state, the deoxyribonucleic acid increases 31-fold in the male and fivefold in the female but ribonucleic acid, which is stored in considerable amount in the eggs but not in the sperms, increases about sevenfold in the male and 27-fold in the female. Protein increases about 17-fold in the male and 28-fold in the female. Lipide (ether-extractable) increases tenfold in the male and 25-fold in the female (94). Cyclic variations of some of these constituents have been studied monthly in *S. purpuratus* (94) and in *S. franciscanus* (78).

In the starfish, *P. ochraceus*, the changes in several of the biochemical constituents were followed over an annual cycle which included monthly sampling (78). In this species a reciprocal relationship exists between the gonads and the gastric caecae. When the gonads have become inactive after spawning, which generally occurs in May, the caecae grow to their largest size. When the gonads begin to grow in late winter and early spring, the caecae begin to dwindle. The caecae of the starfish store considerable lipid, which constitutes about 30 per cent of the dry weight in shrunken caecae and about 50 per cent in caecae of maximal size. Protein content of the caecae does not vary extensively, constituting about 20 per cent of the dry weight of the tissue at all times. The content of glycogen in caecal tissue is never large (1.7 per cent). Spent ovary of the starfish has little lipid—about 5 per cent of its dry weight, while gravid ovary has much more—about 30 per cent. Spent (or immature) testis has about 2 per cent lipid by dry weight lipid, gravid about 18 per cent. Protein and nonprotein nitrogen are generally found in rather constant proportion in the gonads, constituting 43 and 3.5 per cent of the dry weight, respectively. Glycogen, being here a

minor constituent (0.35 per cent), is perhaps for that reason of lesser interest although it varies from time to time (78). Somewhat similar results were also obtained for another starfish, *P. giganteus* (78).

Anderson (95) in a study of *Asterias forbesi* points out that the fat reserves seen in the sections of the caecae disappear after six to eight weeks of starvation, although the zymogen cells and the mucus-secreting cells are still functional. Histochemical tests, therefore, confirm the conclusions reached on the basis of chemical determinations (78). Karnovsky *et al.* (96), using the same species, fractionated the lipide and showed the presence of phosphatides, steroids, aldehyde lipides, and neutral fats.

Considerable glycogen (up to 11.6 per cent) is stored in the gonad of the sea urchin, *Echinus esculentus* (18), in contrast to relatively small amounts in *S. purpuratus* (94). The glycogen content of the gonad declines just before the gonads mature. Summarizing data gathered by other investigators on glycogen content of oysters and mussels, Stott (18, p. 148), concludes.

... in oysters in the Whitstable beds spawning begins usually about the middle of June, so there again is a glycogen minimum prior to spawning. The succession of times of onset of spawning is, therefore, in the order *Mytilus*, *Echinus*, *Ostrea*, which is the order of occurrence of glycogen minima.

Russo (97, 98) followed the nitrogen and amino acid content of gonads of the urchins, *S. lividus*, and found variations in their content. It is apparent that quantitatively the lipides are of extreme importance to the economy of many echinoderms and that there is some likelihood of storage of lipides, early in the season, as reserves for the reproductive process. In the starfishes, where pyloric caecae are present, these serve as depositories but in the urchins without such organs most of the storage occurs in the gonads themselves.

INDUCTION OF SPAWNING IN MARINE INVERTEBRATES

Because spawning is such a dramatic act it has been described in detail in many species, and many factors and relationships which insure synchrony of spawning have been of interest to a great number of investigators. The literature, therefore, is quite voluminous. Spawning of course implies that the animals are in mature physiological state and that the gametes discharged are ripe although in some cases, under abnormal conditions, a strong disturbance may lead to discharge of immature cells (22).

Many factors have been considered as playing a part in spawning: temperature change, calm waters after a period of disturbed waters (as by recession of the tide), disturbance after a period of calmness, pressure change, physiological change, nutritional change, illumination change (light after darkness or darkness after light), and chemical influences. A picture of the possible mechanism of spawning from all these results is confusing, perhaps because of a search for a single factor inciting spawning. Rather, it is more probable that several factors, working simultaneously or in succession, may be of importance, especially some physical factor and a chemical factor following it.

A change in temperature has long been considered by many workers as the most important single factor incitant to spawning (17, 19, 67, 84, 105, 106, 129 to 132). The limpet, *Patella vulgata*, in Plymouth waters spawns at the time of maximum and minimum temperature (84). The mussel, *Mytilus galloprovincialis*, may spawn in autumn with falling, or in spring with rising temperature (103). The mussel, *M. edulis*, on the Atlantic Coast of North America begins to spawn with onset of warmer conditions (104). For the mussel, *M. californianus*, it is claimed by some (104) that spawning comes with the cooling of the waters, but Young (88, 89) could find no correlation between temperature, or temperature change, and spawning in this species. However, correlation between temperature change and spawning has been indicated in a number of species (148, 155, 156). A change in temperature, reaching a certain minimal temperature, has been considered necessary for spawning of some species of oyster (17, 19, 84). Loosanoff & Davis (139), on the other hand, showed that gametogenesis in the American oyster, *C. virginica*, did not occur below 10°C. but that at temperatures of 15°C. or higher it occurred, more rapidly the higher the temperature. Thus a winter oyster kept at 30°C. developed some eggs and sperm on the third day and spawned on the fifth. When American oysters from various localities were brought to Milport Harbor, their gonads developed but spawning did not occur at the same temperature, the ones from the north spawning first (138) indicating that the sensitivity of spawning to temperature is probably genotypic. Working with the same species of oyster, *C. virginica*, Galtsoff showed that changes from 20°C. to 22.4, 25, 27.5, 30, and 32.5°C. in each case caused spawning, most readily in the last instance (106). On the other hand, since the oyster is not likely to be subjected to such high temperatures in nature and generally spawns at temperatures lower than these, sometimes even at quite low temperatures (107), this information is, perhaps, primarily of academic interest in showing the effect of temperature on these physiological processes. After reviewing the extensive literature on the physiology of the oyster, Korrington (82) comes to the conclusion that temperature has some secondary influence on the growth and development of the gonads.

It was found that chitons of a variety of genera and species spawn when isolated in tidepools (14, 15) and that, when disturbed by wave action, they stop spawning. The Atlantic palolo worm only spawns in calm waters—even the physical shock from waves raised by an eight-mile wind will stop their spawning (99).

On the other hand, wave action or other physical shock⁷ stimulates spawning in some animals. It was found that the mussel, *M. californianus*, spawned when mechanically shocked by scraping and byssus-pulling or even

⁷ Shock by injection of isotonic potassium chloride into the sea urchin *Arbacia* (46) and the mussel, *M. edulis* (134), induces spawning and has been used as a means for sexing urchins (23). Electric shock is also successful in mussels (133) and in sea urchins (135). These are hardly likely to play a role in natural spawning but serve to indicate the effect of shock.

by turbulent waters after a period of calm waters (88, 89). Mechanical shock also seems to be of importance to the clam, *Cummingia*, so that sexually mature individuals, just collected, spawn when placed in sea water in the laboratory (16). However, this could not be considered natural stimulation. Fox (49, 50) points out that many sea urchins when collected often spawn, presumably from shock. When the test (shell) is cut or when an exposed ovary is stroked with a brush, spawning is induced. The limpet, *P. vulgata*, appears to spawn during a period of strong shore winds and rough seas (101).

The possible correlation of spawning with phases of the moon has been recently reviewed by Korringa (102) for about two dozen species of marine invertebrates belonging to the Coelenterata, Annelida, Mollusca, and some insufficiently documented cases for representatives of several phyla. Some species show periodicity twice in a lunar cycle, while other species spawn only once during a lunar cycle.

When a bimonthly lunar spawning rhythm asserts itself, it is thought that the tidal shift of a meter or more height of water, which is accompanied by a pressure change, affects the animals and is an incitant to spawning, rather than the light from the moon (102). An example is the European oyster, *O. edulis*. Spawning records of this oyster kept over a period of 20 years show that some spawning occurs every day during the spawning season (June to August) but maximal spawns of greater or lesser importance are observed each year during the two monthly spring tides of June, July, and August. These maxima seem to be uninfluenced by the actual water temperatures or the temperature during the period just preceding spawning. At least seven other species, six annelids (polychaetes) and another mollusk show such lunar (tidal) rhythm (102). Unfortunately, no one has as yet put the suggestion of induction of spawning by pressure change to a test, but experiments are imminent in Korringa's laboratory [see also (169)].

When a monthly spawn is correlated with one phase of the moon only, it is assumed that light rather than pressure change incites the spawning act since two sets of pressure changes occur each month accompanying the two spring and neap tides. Such lunar rhythms have been observed in about a dozen species of animals [Annelida (Polychaeta), Mollusca, Coelenterata] (102). The best known examples of this group of animals are the palolo worms of the Atlantic [*Eunice* (*Leodice*) *fucata*] and of the Pacific [*Eunice* (*Leodice*) *viridis*], upon which an extensive literature has appeared [see Korringa (102) for references].

Clark & Hess (99) give a vivid account of spawning in the Atlantic palolo. The worm differentiates into an atokal (vegetative) and an epitokal (reproductive) portion, the whole worm being negative to light. During the period of swarming the mature epitokal part, now becoming positive to light, breaks loose from the atokal part (which remains in its burrow) and swims as a separate individual to the surface, at 3:00 to 4:00 a.m. The positive reaction to light brings the epitokes together at the surface of the sea, the number of swimmers increasing until dawn, at which time the eggs and sperms are released. The epitokes then die. Reproduction appears not to be limited to a

few days but may occur at random and sporadically for a period of most of the month. However, a plot of the numbers of worms present at the surface shows a distinct and unquestionable peak usually during the third quarter of the moon. Swarming may occur over a wide area almost simultaneously. The males come out sooner than the females and being more fragile they shed first. Shedding of gametes from specimens studied in the laboratory occurs more rapidly in the light than in the dark, so it is presumed that light acts as an incitant (99). The fact that delayed shedding will occur even in the dark indicates that at the end of a cycle of maturity other excitants will work also, though not as quickly as light (99). If the worms in a floating live car are shaded the night before the expected swarm, they will still swarm and spawn (149) but not if they are so shaded for two days or more (108). Something may be built up in the worm itself by the successive exposures to light which enables them to spawn. Since some of the worms in a floating live car spawn at the same time as worms in the sea around them, tidal influence seems of lesser importance although it is always possible that during the short span of the experiment a memory of the tides exists here as it does in the animals studied by Brown and his collaborators (109, 144). Korringa (102) thinks that further experiments are desirable to eliminate the possible influence of the tides and their coincident pressure influences on spawning, even in cases which involve spawning correlated with only one phase of the moon.

Hauenschild (77, 124), in his experiments on the polychaete, *Platynereis dumerilii*, has attempted to show that the light from the moon is a factor in the incitement of spawning in some animals with a single monthly spawn. This worm has been grown in the laboratory for more than ten generations and shows cyclic swarming from January to April and from September to December, with a minimum occurring between 10 and 20 days after the new moon. He found that if he reduced the illumination of the moonlight the swarm cycle was less striking. When he illuminated them continuously with artificial light, swarming was no longer cyclic. Moreover, he found that if he illuminated the worms with artificial light in such a way that he simulated a condition of full moon, when in actuality the moon in the sky was essentially dark, he could get a phase shift in the spawning cycle and the worms now showed spawning activities in phase with the newly imposed light regime. He concludes that it is the period in hours of exposure to light—as a summation of daylight, artificial light, or even light as weak as moonlight—and not the total dosage of light received, which affects the rate of metamorphosis and maturation of the worms, readying them for spawning which occurs several days later. It is intriguing that exposure to moonlight (or artificial light) during the lengthening days of early spring or during the shortening days of late fall should in both cases induce swarming.

The effect of daylight on spawning, studied in several coelenterates and one tunicate, again indicates the action of light as an incitant. In the hydroid, *Pennaria tiarella*, many workers reported that spawning occurs several hours after sunset. If specimens are kept in the laboratory in the dark for 10 to 14 hours and then exposed even for a brief time to light, they will spawn after a

latent period (110, 111). Spawning will recur if the darkness-light regime is repeated but not if illumination is continuous. Ballard (111) suggests that some light-sensitive substance is accumulated in the dark which upon photolysis activates the maturation of gametocytes, leading to spawning. *Hydractinea echinata* in nature appears to spawn at sunrise (111). In this case, spawning is not induced after exposure to light, followed by one to several hours of darkness, but only after a second exposure to the light. Males shed 50 min. after reillumination, females 55 min. after reillumination. One can, therefore, keep specimens in the dark until such time as spawned gametes are needed. Similar observations were made with the ascidian, *Styela partita* (113). Yoshida, working with the hydromedusa, *Spirocodon saltatrix* (112) and with *Hydractinea epiconcha* (159), finds that in these cases ripening of the gonophores takes place in the light and discharge of the gametes occurs only after the gonophores are placed in darkness for a sufficient length of time. In other words, they show a diurnal rhythm in the ripening and spawning of gonophores in the breeding season. Photosensitivity in all these forms probably serves to initiate spawning at a favorable time. Because of differences in sensitivity or of maturity of different reproductive units in the animal, spawning is prolonged over a span of several days or weeks, some responding to light on the first day, others on the second, etc. Only one attempt has been made to define the nature of the pigment mediating the spawning response, the action spectrum suggesting a porphyrin (70).

The effect of chemical incitement of spawning and synchronization of spawning by chemical and hormonal influences has been of interest to many investigators. Myazaki (114) claims that a substance contained in green algae induces spawning of the male oyster. Nelson (115) reviews some of the literature bearing on this problem. Galtsoff (106, 116, 117) demonstrated that sperms and testes of male oysters contained a substance which initiates spawning in the female oyster. The substance is alcohol- and benzene-soluble but not water-soluble. The spawning of the females in turn induces spawning in the more refractory males. They in turn induce spawning in more females, leading by chain reaction to a spawn of epidemic proportions. Male oysters seem to respond also to a variety of hormones and other chemicals as well as to eggs of a variety of mollusks and even of starfishes. Initiation of spawning in males by such extraneous stimuli appears to have survival value because it initiates spawning in the population. In the presence of appropriate sex stimulation, lower temperatures are adequate to initiate spawning in oysters, which seems to indicate that chemical stimulation plays a more dominant role than temperature, which is only a conditioner (106, 116, 117). A second chemical substance (diantlin, a water soluble substance which consists of, or is bound to, nucleic acid and protein in a conjugate, since neither substance alone is active) (115), was found to facilitate the passage of eggs through the ostia of the female oyster by causing enlargement of the pores and increasing the rate of ciliary action. A substance with this effect was obtained from the phytoplankton in one case.

Several other cases of chemical co-ordination and synchronization of "spawning" (which in barnacles may mean release of gametes or release of larvae) have been described. For example, the barnacle, *Balanus balanoides*, spawns in response to the diatom outburst, as happens also in other species in the Arctic where the diatom outburst is of great moment in view of the short favorable developmental time (118). During the more active feeding, following a diatom "bloom", the greater cirrus activity results in solution of the lamellar material which binds the eggs of the barnacle together. An extract of diatom (*Skeletonema costatum*) concentrate was found to have the same effect on the lamella but it was not as effective as the extract of prosomas of the barnacles (118). Another example is the spawning response of female mussels to a substance from the male mussel. In *M. californianus* stimulation by the extract of ground tissue is more potent than mechanical shock. Whatever diffusible substance brings on spawning, it is not normally washed from the gonadal tissue until the tissue is cut, since uncrushed mussels do not elicit the response although the water passes over the exterior of the gonads (88, 89). Interestingly enough, gonad substance incites spawning of the male gonad but not the female gonad in hermaphrodite tridacnid clams (156). Also, in many chitons, isolated females do not spawn even for six to eight weeks after the breeding season (14). However, when males are present they spawn [see also (146)] but the females appear to give up their eggs even before one can see the sperms coming into their vicinity (14).

As was previously discussed, in the Atlantic palolo worms an interplay occurs between males and females which brings about co-ordinated spawning (99). It is possible that the females excited to spawn by the early-spawning males excite remaining males, previously refractory, to shed, and their spawn in turn affects still other females, the chain of reactions leading to epidemic spawning. A similar condition is found for the annelid worms, *Nereis limbata* (119), and *Arenicola clapedii* (40). Strangely enough, in a closely related form, *A. cristata*, the females laid their eggs in the mud before the males shed the sperms (4). In *Platynereis dumerilii*, it appears that the female excites the male to spawn, since isolated females spawn spontaneously while males require the presence of females (124).

Spawning in some sea urchins is thought to be synchronized by chemical or hormonal agents. Fox (50) found that *E. esculentus* females respond to sperm water and males to egg water. Males always react first; therefore in their presence females soon spawn. This has been reported for some West Coast sea urchins (8). Undoubtedly, still other cases exist but the few given illustrate the widespread presence of such relationships.

POSSIBLE MECHANISMS OF INCITEMENT AND CO-ORDINATION OF REPRODUCTION IN MARINE INVERTEBRATES

On the basis of the data reported in this review one might venture a few speculations on possible mechanisms of incitement and co-ordination of reproduction. Because so many quite different physical and chemical factors

can incite spawning in marine invertebrates, it seems likely that each of these acts as a trigger upon a nervous mechanism which calls into action the appropriate responses. On this basis it seems rational that the spawning response should depend both upon internal conditions (such as ripeness) and external conditions, one factor modifying the sensitivity to another as indeed occurs in the various experiments already described. It is also reasonable that some individuals in a population with the lowest threshold to inciting stimuli should act as sparks to set off the remainder of the population by hormonal stimulation. By this means spawning in a large population can be co-ordinated and become epidemic.

Studies on nervous and hormonal interrelationships in the breeding processes of marine invertebrates have been rather few. Lubet (120, 121) using the mussel, *Mytilus edulis*, and the clam, *Chlamys varia*, showed that the maximal spawning reaction to external stimulation corresponds to the period of evacuation of products of the cerebral (and visceral) neurosecretory cells. The secretion is produced just before gametogenesis and is maximal at the time of gamete maturation. Just before each release of gametes (spawning) some of the neurosecretory cells empty their secretions. Lubet removed the cerebral ganglion and found that spawning was hastened. He therefore concludes that the neurosecretion has an inhibitory effect on spawning and that only after the secretions are emptied does the bivalve become sensitive to the environmental factors known to provoke spawning.

Durchon (122, 123) reports a rather similar set of events in some polychaetes. In heteronereid species, removal or suppression of the brain leads to premature epitoke development, irrespective of the age of the individual or its sex. When the prostomium, containing the brain, is transplanted to brainless worms, normal development ensues. The brain thus seems to exercise an inhibitory action upon maturation of spermatozoa and genitalia. The humoral factor is located in the neurosecretory cells of the brain but its method of translocation to the epitoke or gonads is unknown. Some evidence also exists to indicate a positive factor leading to premature epitoke development, which can be initiated by intracoelemic injection of extracts from immature genitals (123).

Two tunicates, *Phallusia mammilata* and *Ciona intestinalis*, respond to vertebrate gonadotropin by release of gametes as they do also by exposure to eggs or sperm of their own species. If the neural gland is destroyed they still respond to gonadotropin but not to eggs or sperm; they respond to neither when the connection from the ganglion to the gland is severed. Carlisle (141) postulates that the sense receptors react to the gametes and, by way of the nervous system, bring about the secretion of a gonadotropin which causes spawning.

Perhaps less pertinent to the immediate discussion, but of interest, are Chariaux-Cotton's (140) experiments on endocrine determination of secondary sex characters in the amphipod, *Orchestia gamarella*. Experimental proof is provided that these characters are controlled by sex hormones.

In passing, it is important to call attention to the fact that behavioral responses bearing upon reproduction may precede incitement of spawning, for example, shoreward migration and aggregation of some sea urchins (18, 158), aggregation of ophiuroids, sea urchins, crabs, sea slugs, and other mollusks (142), and swarming of some polychaete worms (99). Even more complex responses bring about aggregation and pairing of animals which transfer sperms in mating. In these cases the mature gonads may secrete substances which alter the behavior of the animals in response to stimuli from the external environment, causing them to migrate, aggregate, pair up, etc. This type of behavior has not yet been analyzed.

CONCLUSIONS AND PROSPECTS

The review of the literature shows that while some stimulating experimental work has been done on breeding cycles of marine invertebrates, much of it is descriptive. The growth and maturation of gonads and spawning are correlated with environmental factors, but often adequate experimental proof of the generalization drawn from such correlations is lacking. Growth and maturation of gametes are probably influenced more by temperature than by any other factor, there being at present little evidence of photoperiodic (daylength) control of annual reproductive cycles of marine invertebrates, although photoperiodism is not excluded, and perhaps plays a role in some (124). There is as yet no conclusive experimental evidence of chemical control of initiation and growth of gonads in the annual reproductive cycle although it is also not excluded as a possibility. Spawning seems to be initially incited in a population by the action of a variety of stimuli: light, tidal shift, shock, or quietness after shock, temperature change, and chemicals.

Receptors signalling the nervous system are probably involved in incitement of growth and development of the gonads as well as in exciting the spawning reaction. Hormones probably play a role in these reactions and in the spread of a spawning epidemic.

Prospects in the field of reproductive physiology of the marine invertebrates are very good (143). It is clear from the previous discussions that much interesting work has been done and a great deal more has begun. But there is also a tremendous amount still undone which can provide a lifetime of problems for those who are challenged by them.

LITERATURE CITED

1. Prosser, C. L., Ed., *Comparative Animal Physiology* (W. B. Saunders Co., Philadelphia, Pa., 888 pp., 1950)
2. Borradaile, L. A., and Potts, F. A., with chapters by Eastham, L. E. S., and Saunders, J. T., 3rd ed. *The Invertebrata, A Manual for the Use of Students* (Cambridge University Press, London, Engl., revised by Kevkut, G. A., 795 pp., 1958)
3. Hyman, L. H., *The Invertebrates* (McGraw-Hill Book Co., Inc., New York, N. Y., I, 1940; II, III, 1951; IV, 1955)

4. Spector, W. S., *Handbook of Biological Data* (W. B. Saunders Co., Philadelphia, Pa., 584 pp., 1956)
5. Costello, D. S., Davidson, M. E., Eggers, A., Fox, M. H., and Henley, C., *Methods for Obtaining and Handling Marine Eggs and Embryos* (Marine Biological Laboratory, Woods Hole, Mass., 247 pp., 1957)
6. Graham, D. H., *Trans. Proc. Roy. Soc. New Zealand*, **71**, 152-59 (1941)
7. Rickets, E. F., and Calvin, J., *Between Pacific Tides* (Stanford University Press, Stanford, Calif., 3rd ed. Revised by Hedgepeth, J. W., 502 pp., 1952)
8. MacGinitie, G. E., and MacGinitie, N., *Natural History of Marine Animals* (McGraw-Hill Book Co., Inc., New York, N. Y., 473 pp., 1949)
9. Hewett, W. G., *Proc. Calif. Acad. Sci.*, **23**, 283-88 (1938)
10. Costello, D. P., *J. Morphol.*, **63**, 319-43 (1938)
11. Marshall, S. M., and Stephenson, T. A., *Great Barrier Reef Expedition 1928-29, Sci. Repts.*, **3**, 219-45 (1933)
12. Stephenson, A., *Great Barrier Reef Expedition 1928-29, Sci. Repts.*, **3**, 247-72 (1934)
13. MacGinitie, G. E., *Publ. #4221, Smithsonian Misc. Collections*, **128**, 1-201 (1955)
14. Heath, H., *Zool. Anz.*, **29**, 390-93 (1905)
15. Grave, B. H., *Biol. Bull.*, **42**, 234-56 (1922)
16. Grave, B. H., *Biol. Bull.*, **52**, 418-35 (1927)
17. Nelson, T. C., *Ecology*, **9**, 145-54 (1928)
18. Stott, F. C., *J. Exptl. Biol.*, **8**, 133-50 (1931)
19. Hopkins, A. E., *Ecology*, **17**, 551-66 (1936)
20. Young, R. T., *Ecology*, **23**, 490-92 (1942)
21. Young, R. T., *Ecology*, **26**, 58-69 (1945)
22. Hargitt, C. W., *Am. Naturalist*, **44**, 376-78 (1910)
23. Harvey, E. B., *The American Arbacia and Other Sea Urchins* (Princeton University Press, Princeton, N. J., 298 pp., 1956)
24. Moore, H. B., *J. Marine Biol. Assoc. United Kingdom*, **19**, 869-85 (1934)
25. Loosanoff, V. L., *Biol. Bull.*, **72**, 389-405 (1937)
26. Vevers, H. G., *J. Marine Biol. Assoc. United Kingdom*, **28**, 165-87 (1949)
27. Chipperfield, P. N. J., *J. Marine Biol. Assoc. United Kingdom*, **32**, 449-76 (1953)
28. Loosanoff, V. L., *Biol. Bull.*, **104**, 146-55 (1953)
29. Orton, J. H., Southward, A. J., and Dodd, J. M., *J. Marine Biol. Assoc. United Kingdom*, **35**, 149-76 (1956)
30. Churchill, E. P., *U. S. Bur. Fisheries Bull.*, Doc. No. 870, **36**, 95-128 (1918)
31. Broekhuysen, G. J., *Arch. néerl. zool.*, **2**, 257-399 (1936)
32. Broekhuysen, G. J., *Trans. Roy. Soc. S. Africa*, **28**, 331-66 (1941)
33. Hiatt, R. W., *Pacific Sci.*, **2**, 135-213 (1948)
34. Boolootian, R. A., Giese, A. C., Farmanfarmaian, A., and Tucker, J. (Unpublished data)
35. Lasker, R., and Giese, A. C., *Biol. Bull.*, **106**, 328-40 (1956)
36. Bennett, J., and Giese, A. C., *Biol. Bull.*, **109**, 226-37 (1955)
37. Kowalski, R., *Kiel. Meeresforsch.*, **11**, 201-13 (1955)
38. Boolootian, R. A., and Giese, A. C. (Unpublished data)
39. Loeb, J., *Am. Naturalist*, **49**, 257-85 (1915)
40. Okuda, S., *Annotationes Zool. Japan.*, **17**, 577-80 (1938)
41. Just, E. E., *Biol. Bull.*, **27**, 201-12 (1914)
42. Dunbar, M. J., *J. Animal Ecol.*, **9**, 215-26 (1940)
43. Dunbar, M. J., *Canad. J. Research*, **19D**, 258-66 (1941)

44. Thorson, G., *Biol. Revs. Cambridge Phil. Soc.*, **25**, 1-45 (1950)
45. Farmanfarmaian, A., Giese, A. C., Boolootian, R. A., and Tucker, J. S., *J. Exptl. Zool.* (In press)
46. Palmer, L., *Physiol. Zool.*, **10**, 352-67 (1937)
47. Herrington, W. C., *Calif. Fish and Game Fish.*, No. **18**, 1-67 (1930)
48. Koehler, O., *Z. Induktive Abstammungs- u. Vererbungslehre*, **15**, 1-295 (1915)
49. Fox, H. M., *Proc. Roy. Soc. London*, **B95**, 523-50 (1924)
50. Fox, H. M., *Proc. Cambridge Phil. Soc. Biol. Sci.*, **1**, 71-74 (1924)
51. Atoda, K., *Sci. Repts. Tôhoku Univ., 4th Ser.*, **20**, 105-21 (1953)
52. Paul, M. D., *Proc. Indian Acad. Sci.*, **B15**, 1-42 (1942)
53. Aiyar, R. G., *J. Madras Univ.*, **5**, 115-50 (1933)
54. Pannikar, N. K., and Aiyar, R. G., *Proc. Indian Acad. Sci.*, **B9**, 343-54 (1939)
55. Talvera, F., and Faustino, L. A., *Philippine J. Sci.*, **50**, 1-48 (1933)
56. Yonge, C. M., *Pap. Tortugas Lab. Carnegie Inst. Wash.*, **29**, 185-98 (1935)
57. Yonge, C. M., *A Year on the Great Barrier Reef* (Putnam, London, Engl. and New York, N. Y., 246 pp., 1930)
58. Coe, W. R., *Biol. Bull.*, **71**, 122-32 (1936)
59. Boolootian, R. A., Giese, A. C., Tucker, J. S., and Farmanfarmaian, A., *Biol. Bull.* (In press, 1959)
60. Runnström, S., *Bergens Museums Årbok. Naturv. Rekhe* No. **3**, 1-36 (1936)
61. Moore, R. F., *Rept. U. S. Comm. Fisheries for 1905*, Doc. No. 610, 1-86 (1905)
62. Nelson, T. C., *Ecology*, **9**, 145-54 (1928)
63. Korringa, P., *Année biol.*, **33**, 1-17 (1957)
64. Baird, R. H., and Gibson, F. A., *J. Marine Biol. Assoc. United Kingdom*, **35**, 555-62 (1956)
65. Weymouth, F. W., McMillin, H. C., and Holmes, H. B., *U. S. Bur. Fisheries Bull.* **41**, Doc. No. 984, 201-36 (1925)
66. Marshall, S. M., and Orr, A. P., *Année biol.*, **33**, 43-47 (1957)
67. Loosanoff, V. L., and Davis, H. C., *Science*, **115**, 675-76 (1952)
68. Crisp, D. J., *Nature*, **179**, 1138-39 (1957)
69. Feder, H. M., *Natural History Studies on the Starfish, Pisaster ochraceus* (Brandt, 1835) in the Monterey Bay Area (Doctoral thesis, Stanford University, Stanford, Calif., 1956)
70. Hendricks, S. B., and Borthwick, H. A., in *Synthesis and Order in Growth*, 449-69 (Rudnick, D., Ed., Princeton University Press, Princeton, N. J., 150 pp., 1954)
71. Bullough, W. S., *Vertebrate Sexual Cycles* (Methuen & Co., Ltd., London, Engl., 117 pp., 1951)
72. Lees, A. D., *The Physiology of Diapause in Arthropods* (Cambridge University Press, London, Engl., 151 pp., 1955)
73. Hughes, E., *J. Marine Biol. Assoc. United Kingdom*, **24**, 543-47 (1949)
74. Imai, T., Hatanaka, M., Sato, R., and Yuki, R., *Tôhoku. J. Agr. Research*, **1**, 69-86 (1950)
75. Coe, W. R., *Biol. Bull.*, **75**, 274-85 (1938)
76. Crisp, D. J., and Davies, P. A., *J. Marine Biol. Assoc. United Kingdom*, **34**, 357-80 (1955)
77. Hauenschild, C., *Naturwissenschaften*, **41**, 556-57 (1954)
78. Greenfield, L., Giese, A. C., Farmanfarmaian, A., and Boolootian, R. A., *J. Exptl. Zool.* (In press)
79. Boolootian, R. A. (Personal communication)

80. Coe, W. R., *Science*, **74**, 247-49 (1931)
81. Coe, W. R., *Biol. Bull.*, **71**, 353-59 (1936)
82. Korringa, P., *Quart. Rev. Biol.*, **27**, 266-308, 339-65 (1952)
83. Nelson, T. C., *Am. Scientist*, **45**, 301-32 (1957)
84. Orton, J. H., *J. Marine Biol. Assoc. United Kingdom*, **12**, 339-66 (1920)
85. Loosanoff, V. L., *Biol. Bull.*, **72**, 406-16 (1937)
86. Loosanoff, V. L., and Nomejko, C. A., *Ecology*, **32**, 113-34 (1951)
87. Hsaio, S. C. T., *Biol. Bull.*, **76**, 280-303 (1939)
88. Young, R. T., *Ecology*, **23**, 490-92 (1942)
89. Young, R. T., *Ecology*, **26**, 58-69 (1946)
90. Daniel, R. J., *Liverpool Univ. Lancs. Sea-Fisheries Lab. Rept. No. 31*, 27-50 (1923)
91. Calderwood, H. N., and Armstrong, A. R., *J. Assoc. of Offic. Agr. Chemists*, **24**, 154-64 (1941)
92. Hatanaka, M., *Bull. Japan. Soc. Sci. Fisheries*, **9**, 21-26 (1940)
93. Humphrey, G., *Australian J. Exptl. Biol. Med. Sci.*, **19**, 311-12 (1941)
94. Giese, A. C., Greenfield, L., and Huang, H., *Biol. Bull.* (In press, 1959)
95. Anderson, J. M., *Biol. Bull.*, **105**, 47-61 (1953)
96. Karnovsky, M. L., Jeffry, S. S., Thompson, M. S., and Deane, H. W., *J. Biophys. Biochem. Cytol.*, **1**, 173-82 (1955)
97. Russo, G., *Boll. accad. Gioenia*, **51**, 39-43 (1923)
98. Russo, G., *Arch. sci. biol. (Bologna)*, **8**, 161-81 (1926)
99. Clark, L. B., and Hess, W. N., *Pap. Tortugas Lab. Carnegie Inst. Wash.*, **33**, 71-81 (1942)
100. Orton, J. H., *Nature*, **114**, 191-92 (1924)
101. Orton, J. H., Southward, A. J., and Dodd, J. M., *J. Marine Biol. Assoc. United Kingdom*, **35**, 149-76 (1956)
102. Korringa, P., *Ecol. Monographs*, **17**, 347-81 (1947)
103. Berner, L., *Bull. inst. oceanog.* No. 680, 1-8 (1935)
104. Field, I. A., *Bull. U. S. Bur. Fisheries*, Doc. No. 922, **38**, 127-259 (1922)
105. Whedon, W. F., *Univ. Calif. Publs. Zool.*, **41**, 35-44 (1936)
106. Galtsoff, P. S., *Biol. Bull.*, **75**, 286-307 (1938)
107. Loosanoff, V. L., and Davis, H. C., *Science*, **111**, 521-22 (1950)
108. Mayer, A. G., *Pap. Tortugas Lab. Carnegie Inst. Wash. Publ. No. 102*, **1**, 105-12 (1908)
109. Brown, F. A., in *Recent Advances in Invertebrate Physiology*, 287-304 (Scheer, B. T., Ed., University of Oregon Publications, Eugene, Ore., 1957)
110. Baker, E. C. C., *Proc. Indiana Acad. Sci.*, **45**, 251-52 (1936)
111. Ballard, W. W., *Biol. Bull.*, **82**, 329-39 (1942)
112. Yoshida, M., *Zool. Mag. (Tokyo)*, **61**, 358-66 (1952)
113. Rose, S. M., *Biol. Bull.*, **77**, 216-32 (1939)
114. Myazaki, I., *Bull. Japan. Soc. Sci. Fisheries*, **7**, 137-38 (1938)
115. Nelson, T. C., *Am. Scientist*, **45**, 301-32 (1957)
116. Galtsoff, P. S., *Biol. Bull.*, **75**, 286-307 (1938)
117. Galtsoff, P. S., *Biol. Bull.*, **78**, 117-35 (1940)
118. Barnes, H., *Année biol.*, **33**, 67-85 (1957)
119. Lillie, F. R., and Just, E. E., *Biol. Bull.*, **24**, 147-68 (1913)
120. Lubet, P., *Ann. sci. nat. Zool. et biol. animale*, Ser. 11, **18**, 175-83 (1956)
121. Lubet, P., *Année biol.*, **33**, 19-29 (1957)

122. Durchon, M., *Ann. sci. nat. Zool. et biol. animale*, Ser. 11, **18**, 269-73 (1956)
123. Durchon, M., *Année biol.*, **33**, 31-42 (1957)
124. Hauenschild, C., *Z. Naturforsch.*, **10B**, 658-62 (1955)
125. Martin, E. A., *Biol. Bull.*, **65**, 99-105 (1933)
126. Hempelmann, F., *Zoologica*, **25**, 1-135 (1911)
127. Coe, W. R., *J. Exptl. Zool.*, **104**, 1-24 (1947)
128. Lysaght, A. M., *J. Marine Biol. Assoc. United Kingdom*, **25**, 41-67 (1941)
129. Loosanoff, V. L., *Ecology*, **18**, 506-15 (1937)
130. Colwin, L. H., *Biol. Bull.*, **95**, 296-306 (1948)
131. Burdon-Jones, C., *J. Marine Biol. Assoc. United Kingdom*, **29**, 625-38 (1950)
132. Yamamoto, G., *Sci. Repts. Tôhoku Univ.*, **20**, 11-32 (1953)
133. Iwata, K. S., *Bull. Japan. Soc. Sci. Fisheries*, **15**, 439-42 (1950)
134. Iwata, K. S., *Bull. Japan. Soc. Sci. Fisheries*, **17**, 91-92 (1951)
135. Harvey, E. B., *Nature*, **173**, 86 (1954)
136. Cole, L. C., *Science*, **125**, 874-76 (1957)
137. Withrow, R. B., Ed., *Symposium on Photoperiodicity in Plants and Animals* (Gatlinburg, Tenn., 1957); Am. Assoc. Advance. Sci., Washington, D. C., in press, 1958)
138. Loosanoff, V. L., and Nomejko, C. A., *Biol. Bull.*, **101**, 151-56 (1951)
139. Loosanoff, V. L., and Davis, H. C., *Biol. Bull.*, **103**, 80-86 (1952)
140. Charniaux-Cotton, H., *Ann. sci. nat. Zool. et biol. animale*, Ser. 11, **18**, 305-10 (1956)
141. Carlisle, D. B., *Biol. Bull.*, **28**, 463-72 (1951)
142. MacKay, D. C. C., *Ecology*, **26**, 205-7 (1945)
143. Deacon, G. E. R., *Nature*, **177**, 353-55 (1956)
144. Brown, F. A., Jr., in *Perspectives in Marine Biology*, 269-82 (Buzzati-Traverso, A. A. Ed., University of California Press, Berkeley, Calif., 621 pp., 1958)
145. Tanaka, S., and Hatana, H., *Publ. Seto Marine Biol. Lab. Kyoto Univ.*, **2**, 341-55 (1952)
146. Crozier, W. J., *Am. Naturalist*, **56**, 478-80 (1922)
147. Hancock, D. A., Drinnan, R. E., and Harris, W. H., *J. Marine Biol. Assoc. United Kingdom*, **35**, 307-25 (1956)
148. Yamamoto, G., *Sci. Repts. Tôhoku Univ.*, **18**, 477-81 (1950)
149. Treadwell, A. L., *Carnegie Inst. Yearbook*, **8**, 150 (abstr.) (1909)
150. Southward, E. C., and Southward, A. J., *J. Marine Biol. Assoc. United Kingdom*, **37**, 267-86 (1958)
151. Crisp, D. I., and Southward, A. J., *J. Marine Biol. Assoc. United Kingdom*, **37**, 157-208 (1958)
152. Menzel, R. W., *Science*, **113**, 319-21 (1951)
153. Rees, C. B., *Bull. Marine Ecol.*, **4**, 21-46 (1954)
154. Rees, C. B., *Bull. Marine Ecol.*, **4**, 47-67 (1954)
155. Ino, R., Sagara, J., Hamada, S., and Tamakawa, M., *Bull. Japan. Soc. Sci. Fisheries*, **21**, 32-36 (1955)
156. Wada, S. K., *Japan. J. Zool.*, **11**, 273-85 (1954)
157. Butler, P. A., *Proc. Nall. Shellfish Assoc.*, **46**, 75 (1956)
158. Lewis, J. B., *Can. J. Zool.*, **36**, 607-21 (1958)
159. Yoshida, M., *J. Fac. Sci., Univ. of Tokyo*, 7 (Part 1), 67-78 (1954)
160. Montalenti, G., in *Perspectives in Marine Biology*, 589-602 (Buzzati-Traverso, A. A., Ed., University of California Press, Berkeley, Calif., 621 pp., 1958)

161. Barnes, H., in *Perspectives in Marine Biology*, 105-16 (Buzzati-Traverso, A. A., Ed., University of California Press, Berkeley, Calif., 621 pp., 1958)
162. Loosanoff, V. L., in *Perspectives in Marine Biology* (Buzzati-Traverso, A. A., Ed., University of California Press, Berkeley, Calif., 621 pp., 1958)
163. Fraga, I. F., *Consejo super. invest. cient., Patronato "Juan de la Cierva" invest. pesquera*, **4**, 109-25 (1956)
164. George, J. C., and Patel, B. S., *J. Animal Morphol. and Physiol.*, **3**, 49-55 (1956)
165. Crisp, D. L., *J. Marine Biol. Assoc. United Kingdom*, **33**, 473-96 (1954)
166. Tanaka, Y., *Bull. Fac. Fisheries Hokkaido Univ.*, **9**, 29-36 (1958)
167. Jennings, R. H., and Whitaker, D. M., *Biol. Bull.*, **80**, 194-201 (1941)
168. Ranade, M. R., *J. Mar. Biol. Assoc. United Kingdom*, **36**, 115-120 (1957)
169. Mileikovskiy, S. A., *Proc. (Doklady) Acad. Sci. U.S.S.R.*, **123**, 564-67 (1958)

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